THE

SCIENTIFIC PROCEEDINGS

OF THE

ROYAL DUBLIN SOCIETY

Vol. 24, N.S., Nos. 25—30 SEPTEMBER, 1948.

25.—AWARD OF THE BOYLE MEDAL To Professor J. H. J. Poole, M.A., M.A.I., Sc.D., 1947.

26.—TRANSFORMATION REACTIONS. I. THE MECHANISM OF THE TRANSFORMATION OF o-AROYLOXYACETOARONES INTO o-HYDROXYDIAROYLMETHANES, AND THE SYNTHESIS OF FLAVONES. By B. G. Doyle, Fionnghuala Gógan, J. E. Gowan, John Keane and T. S. Wheeler.

27.—SEED TRANSMISSION OF VIRUS YELLOWS OF SUGAR BEET (*Beta vulgaris* L.) AND THE EXISTENCE OF STRAINS OF THIS VIRUS IN EIRE. By Phyllis E. M. Clinch and J. B. Loughnane. (Department of Plant Pathology, University College, Dublin). (Plates 13–17).

28.—THE CHEMICAL CONSTITUENTS OF LICHENS FOUND IN IRELAND. *Buellia canescens.* Part 3. THE CONSTITUTION OF DIPLOICIN. By (The late) Thomas J. Nolan, Joseph Algar, Eugene P. McCann, William A. Manahan and Niall Nolan, University College, Dublin.

29.—SALMON AND SEA TROUT OF THE RIVER INNY. By Arthur E. J. Went. (Plate 18).

30.—THE DIAZO REACTION IN THE TETRAZOLE RING. By J. Reilly, J. P. Teegan and M. F. Carey.

[*Authors alone are responsible for all opinions expressed in their Communications.*]

DUBLIN:

PUBLISHED BY THE ROYAL DUBLIN SOCIETY.

BALL'S BRIDGE, DUBLIN.

HODGES, FIGGIS, AND CO.

6 DAWSON STREET, DUBLIN.

WILLIAMS AND NORGATE,

36 GREAT RUSSELL STREET, LONDON, W.C.1.

1948.

Price Fifteen Shillings

COMMONWEALTH MYCOLOGICAL INSTITUTE
17 NOV 1948

Royal Dublin Society.

FOUNDED, A.D. 1731. INCORPORATED, A.D. 1750.

THE Scientific Meetings of the Society are usually held at 4.15 p.m. on the fourth Tuesday of every month of the Session (November to June).

The attention of authors is directed to the following extracts from the regulations governing the presentation of communications at Scientific Meetings of the Society, and the publication of papers in the Scientific or Economic Proceedings:—

> " An author who desires to present a communication at a Scientific Meeting of the Society shall furnish to the Registrar a fortnight previous to that meeting the title and an abstract (not exceeding 200 words) of his communication, together with an estimate of the time required for presentation.
>
> " An author who desires to have a communication published forthwith as a paper in the Proceedings of the Society shall furnish to the Registrar the full text and necessary illustrations at least a week before the meeting of the Committee of Science at which he desires publication to be considered. Meetings of the Committee of Science are usually held on the second Tuesday of each month from November to June inclusive.
>
> " A paper may be published either in the Scientific Proceedings or the Economic Proceedings of the Royal Dublin Society, as directed by the Committee of Science. The copyright of papers so published shall be the property of the Society.
>
> " Fifty copies of each paper shall be supplied free of cost to the Author or Authors.
>
> " Additional copies may be obtained at Cost Price if applied for by the Author when returning the proofs.

Authors are requested to apply to the Registrar for further information concerning the preparation of papers and illustrations for press.

Authors of biological papers involving extensive numerical observations, are requested to consult the recommendations of the British Association Committee on Biological Measurements, 1927, obtainable from the British Association, Burlington House, W.1, price 6d. Arrangements have been made whereby original data, too extensive for complete publication, may be deposited at the British Museum (Natural History), or with the Royal Society of Edinburgh, and so be made available for future workers. Authors desirous of depositing data, whether zoological or botanical, in this manner, should communicate with either the Keeper of Zoology, Natural History Museum, S.W.7, or the General Secretary, The Royal Society of Edinburgh, 22 George Street, Edinburgh, enclosing full bibliographic reference to the publication in which the results are summarised.

No 25.

AWARD OF THE BOYLE MEDAL

To Professor J. H. J. Poole, M.A., M.A.I., Sc.D.

1947.[1]

Report of the Committee of Science and Its Industrial Applications.

Dr. J. H. J. Poole's reputation rests principally on his contributions to the sciences of radio-activity and geophysics, investigations which involve a profound knowledge and technical skill in two or more provinces of scientific advance; indeed, in these matters quite few are qualified to give judgment. His pioneer researches on the distribution of radium and thorium in rocks (partly in collaboration with Joly, but also independently) were of outstanding importance, and for this work alone geologists owe much to Dr. Poole. His papers are critical and constructive and are characterised by a refreshing freedom from dogmatism. The originality of his methods of attack also is noteworthy. His investigation entitled "The Evolution of the Atmosphere" attracts immediate attention, and has been quoted widely with approval. He has recognised new problems, devised new methods, and suggested new hypotheses which are brilliant and stimulating.

[1] The Medal was awarded by the Council on the 10th April 1947, and presented to Professor Poole by the President at the Stated General Meeting of the Society on 13th November, 1947.

No. 26.

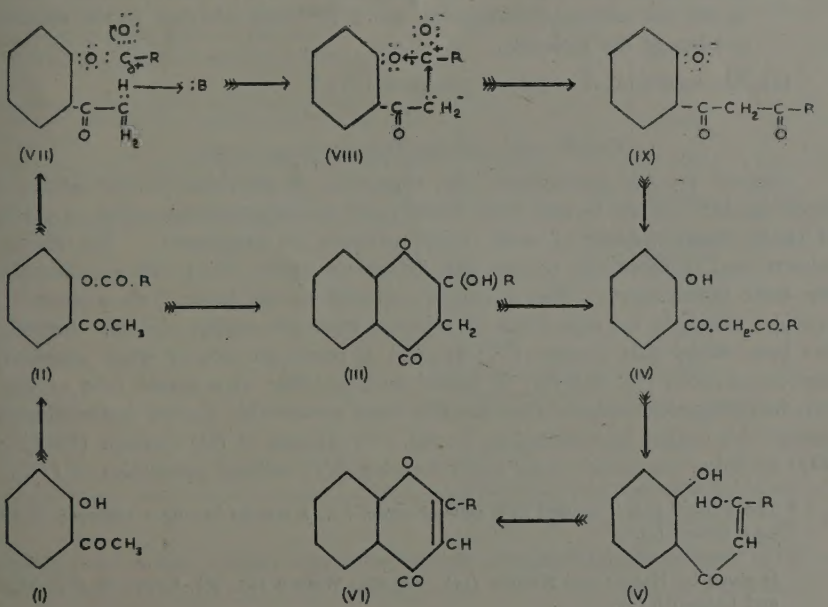
TRANSFORMATION REACTIONS. I. THE MECHANISM OF THE TRANSFORMATION OF *o*-AROYLOXYACETOARONES INTO *o*-HYDROXYDIAROYLMETHANES, AND THE SYNTHESIS OF FLAVONES.

By B. G. Doyle, Fionnghuala Gógan, J. E. Gowan, John Keane and T. S. Wheeler.

[Read January 27, 1948. Published Separately May 26, 1948.]

Introduction

In 1933 Baker (1, 2) found that anhydrous potassium carbonate in hot benzene or toluene, effected the transformation of *o*-aroyloxyacetoarones (II), into the corresponding *o*-hydroxydiaroylmethanes (IV), with, he suggested, the intermediate formation of the hydroxyflavanone (III). The same rearrangement, which is of synthetical importance, owing to the ready and sometimes spontaneous convertibility of (V), the enolic form of (IV), into a flavone (VI), was independently discovered by Venkataraman and his collaborators (5, 14, 15), the reagent used by them being sodamide in dry ether.



Mechanism of the Transformation

Later Virker and Wheeler (24) and Ullal, Shah and Wheeler (21) found that sodium in ether or boiling toluene, and sodium ethoxide in alcohol, were also effective reagents for this transformation, and in view of these results Ullal and Wheeler (20) tentatively suggested, that the reaction might be regarded as an internal Claisen condensation, between an ester and a ketone, differing from the Dieckmann reaction in that the alcohol (in this case a phenol) eliminated remains

part of the molecule undergoing transformation. If this view is correct, the mechanism of the reaction involves a nucleophilic displacement of oxygen on carbon by the carbanion formed by the proton attraction of the basic* transforming agent thus:—

(*a*) The base (:B) removes a proton from the methyl carbon, which being adjacent to the carbonyl group, contains " ionizable " hydrogen (VII).

(*b*) The carbonyl carbon of the ester, which is electrophilic by resonance, tends to accept the lone pair of electrons formed on withdrawal of the proton. As negatively charged carbon approaches negatively charged oxygen recedes, with the intermediate formation of a transition state (VIII), which corresponds to Baker's intermediate compound (III).

(*c*) Elimination of phenol occurs with the production of a diketone, which separates as a salt of the metal ion present (IX). Baker (1) showed that in the salt so formed, the metal ion is probably attached to the diketone portion of the molecule.

(*d*) The addition of a proton produces (IV).†

Results with various Transforming Agents

Support for the mechanism now suggested, is provided by its success in predicting that, as will be seen from Table I and the experimental section, a variety of strong bases (anions of weak acids) produce rearrangement. The reaction solvent used is important; acetone and dioxan are useful, but pyridine is generally the most satisfactory. This is to be expected as the basic " atmosphere " it provides assists in the separation of a proton from the methyl carbon. Indeed it has been found that flavone (VI; R=Ph) is produced directly when *o*-benzoyloxyacetophenone (II; R=Ph) is heated with pyridine in a sealed tube at 275°, *o*-hydroxydibenzoylmethane (IV; R=Ph) being presumably formed intermediately, though this cannot be regarded as settled. Cyclization of (II) through (VIII), or (III) as Baker suggested, could occur forming (VI) without production of (IV).

* The word " base " is used in a general sense, *i.e.*, a species having a tendency to add on a proton (4).

† The theory of the intermolecular Claisen condensation together with a list of reagents is given by Hauser and Hudson (11). See also Watson (25, 26); Lapworth (13); Hann and Lapworth (9).

TABLE I.

Yields obtained in the Transformation of *o*-Benzoyloxyacetophenone (II; R=Ph) into *o*-Hydroxydibenzoylmethane (IV: R=Ph).[1]

Results with alkali metals and their carbonates.							Results with other transforming agents.				
Metal M	Electronegativity	Yields (%) of crude product[2] with M in solvent shown			Yields (%) of crude product[3] with M_2CO_3 in pyridine. pK (H_2CO_3) = 6.5	Time (hrs.) of heating with M_2CO_3 under reflux	Transforming agent	pK of related acid	Reaction solvent	Yield (%) of crude product	m.p. (crude) except where recrystallising solvent is shown[4]
		Pyridine	Acetone	Dioxan							
Li	1·0	48	40	48	No reaction	6	Na salt of (I)	—	Pyridine	80	117–119° (alcohol)
Na[5]	0·9	56	51	62	65	6	KOH[6]	16	Pyridine	83	119·5–120°
K	0·8	69	70	52	74[7]	1	NaOH	16	Pyridine	74	119–120°
Rb	0·8	69	—	52	82	0·5	NaH*	—	Pyridine	72	113–115°
Cs	0·7	—	—	—	75	0·25	NaOPh	10	Pyridine	70	111–115°
							Na_2O_2	12	Pyridine	64	118–119°
							,,	,,	Acetone	42	117–120°
							,,	,,	Dioxan	40	118–119°
							$NaC(Ph)_3$*	33	Ether	64	119–120° (alcohol)
							NaOEt*	18	Pyridine	60	113–116°
							,,	,,	Pyridine–alcohol (3 : 2)	31	119–120°
							NaOMe*	16	Pyridine	43	117–119°
							,,	,,	Acetone	50	117–120°
							,,	,,	Dioxan	48	117–119°
							,,	,,	Alcohol	18	103–110°
							$NaNH_2$*	—	Pyridine	56	117–118°
							,,	—	Dioxan	50	118–119°
							,,	—	Pyridine–triethylamine (3:1)	50	117–119°
							,,	—	Pyridine–acetone (1:1)	54	118–119°
							,,	—	Liquid ammonia	17	119° (alcohol)
							Ethyl sodioacetoacetate	11	Pyridine	50	114–117°
							PhMgBr[8]	—	Ether	15	120° (alcohol)
							MeMgI[8]	—	Ether	8	119° (alcohol)
							Sodium aluminate	—	Pyridine	41	115–116°
							MeCOOK	4·7	Pyridine	24	116–118°
							PhCOOK	4·2	Pyridine	10	120° (alcohol)

1. For reaction conditions see "Experimental."

2. The m.p. of the crude product was not below 115°. Mixed m.p. with (IV; R=Ph) was nearer to 120°.

3. The m.p. of the crude product was not below 118°. Mixed m.ps. were satisfactory.

4. Mixed m.ps. with (IV; R=Ph) were satisfactory. Addition of 2% and of 10% of (II; R=Ph) reduces the initial m.p. from 120° to 117° and 111° respectively.

5. Na in ether gave a yield of 50%(24)

6. KOH gave a 79% yield with stirring for 1½ hours at room temp.

7. K_2CO_3 in toluene gave a yield of 32%(1). If 10% of water is added to the pyridine solvent the yield is greatly reduced.

8. Other Grignard reagents have been used previously in intermolecular Claisen condensations.

*Previously used for intermolecular Claisen condensations.

Alkali metals. Turning to the examination of the semi-quantitative results in Table I, it will be observed that with the alkali metals the yields increases with the basicity of the metals used, lithium giving generally the lowest and rubidium (metallic caesium was not available) the highest yield; the result with sodium in dioxan is anomalous. The electronegativity values (power of attracting electrons) shown in the Table are taken from Pauling (18), the lower this value the greater the basicity of the metal. The fact that a graph of yield of *o*-hydroxydibenzoylmethane against the reciprocal of the electronegativity (Fig. 1.) is a straight line with pyridine and acetone as solvents may be of significance, as the action of the metal in effecting transformation is to be referred to the displacement of hydrogen from the acetyl methyl group to form an ion of the type shown in (VIII).

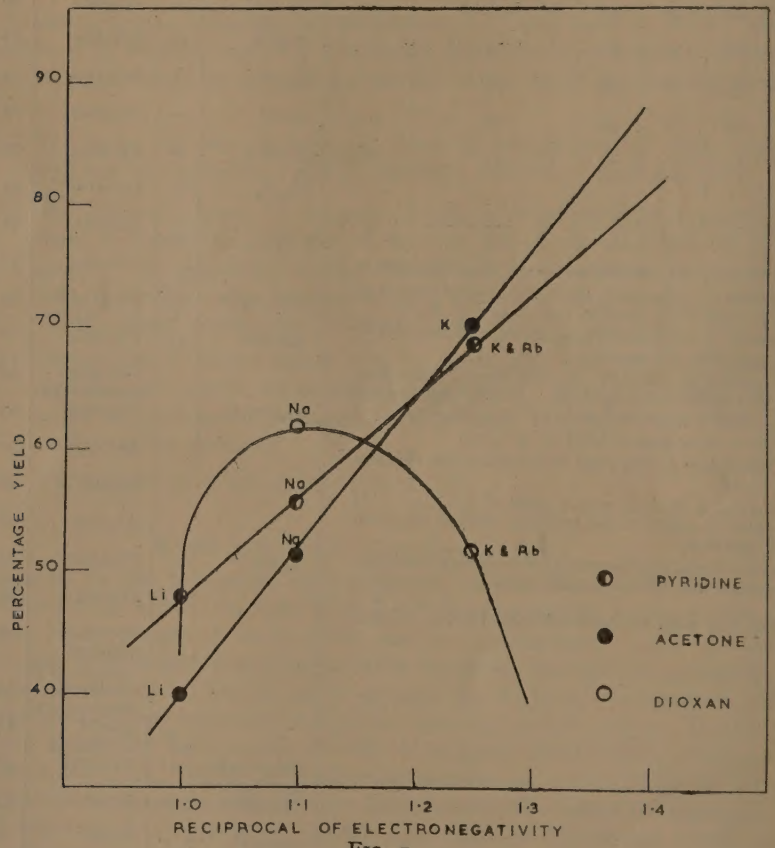


FIG. 1.

Alkali metal carbonates. Here also the yield tends to increase with the basicity of the metal ion. Lithium carbonate is inactive, while with caesium and rubidium carbonates the transformation is rapid, as indicated by the formation of the yellow salt of *o*-hydroxydibenzoylmethane. It should be noted that potassium hydrogen carbonate gives a yield of only 25%, and sodium hydrogen carbonate 10%, over a period of three hours; these salts are much less effective than the normal carbonates.

Other transforming agents. In this section of Table I the approximate pK value for the acid constant of the anion concerned, which is given by

$$K = [OH_3^+][:B]/[H:B]; \quad pK = -\log. K.$$

has been shown, where a figure is available. (See also Fig. 2.). The greater the value of the pK, the weaker the acid and the stronger the conjugate base; the triphenylmethyl anion (pK=33) is one of the strongest bases known. Fig. 2 shows that there is a tendency for yield to increase with pK; the results with the alkoxides have been omitted as they are reduced by alcoholysis.

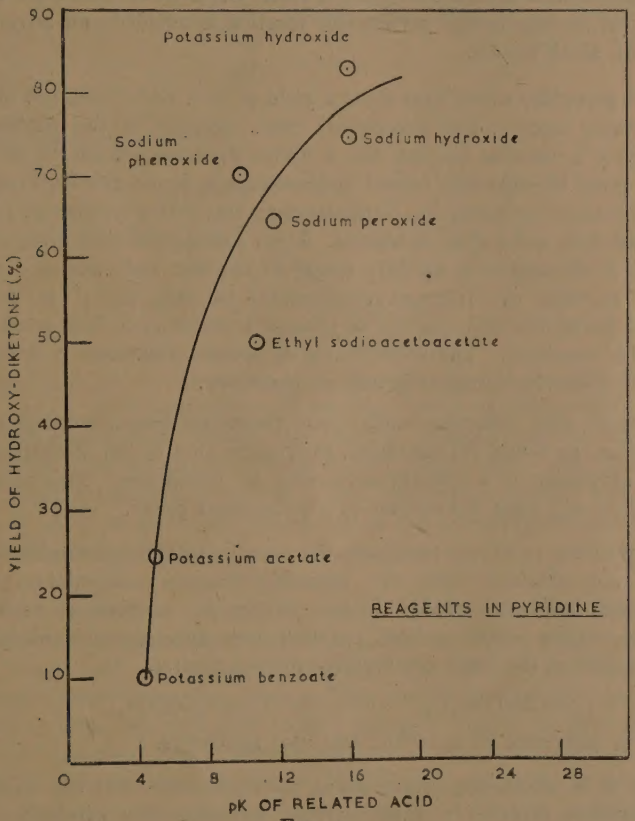


FIG. 2.

It will be observed that some of the effective transforming agents have previously been used in the Claisen intermolecular condensations (See Hauser and Hudson, (11); for the use of sodium hydride see Hansley and Carlisle, (10); Swamer and Hauser, (19)). It is hoped to examine the effect in such inter-molecular condensations, of the other transforming agents here employed.

Taking the results in detail, the high yield obtained with the sodium salt of *o*-hydroxyacetophenone is probably to be attributed to the fact that with it alcoholysis merely reforms *o*-benzoyloxyacetophenone, the ester undergoing transformation. The relatively low yields obtained with alkoxides, especially in the presence of alcohol, can be referred to alcoholysis—this phenomenon is discussed

below in connection with nitro-esters. Ullal, Shah, and Wheeler, (21), however, found sodium ethoxide in alcohol a useful transforming agent with esters of hydroxyacetonaphthones. Sodium phenoxide, where presumably there is less tendency to alcoholysis, gave a good yield in the present instance. The smooth transformation obtained with anhydrous sodium and potassium hydroxides may be linked with the basic strength of hydroxylion. The value of sodium hydride in Claisen condensations is, to some extent, due to the fact that the hydrogen anion ($:H^-$) combines with the proton (H^+) it removes, to form molecular hydrogen, which escapes, so that further undesirable reaction is avoided—an advantage also obtained with alkali metals.

Sodium peroxide, which here gives a yield of over 60%, does not often figure as a condensing agent. The exceptional basic strength of the triphenylmethyl anion makes it a valuable reagent, but it leaves triphenylmethane to be removed. The yields given by sodamide (about 50%, except in liquid ammonia) are greater than those obtained by Mahal and Venkataraman (15) with a number of 1-aroyloxy-2-acetonaphthones, using ether as solvent. Ethyl sodioacetoacetate gives a moderate yield: with it alcoholysis is unlikely owing to the size and resonance of its ion. The basic strength of Grignard compounds ($R^{-+}Mg^+Hal^-$) is due to the tendency of the hydrocarbon radical to acquire a proton, but their great reactivity produces side reactions. The effectiveness of sodium aluminate is interesting, as it seldom functions as a reagent in organic chemistry.

The results with potassium acetate and potassium benzoate indicate that this transformation, as Baker (1) suggested, provides a clue to the mechanism of salt-anhydride aroylation of *o*-hydroxyacetoarones to chromones. This aspect of the transformation will form the subject of a subsequent paper.

Using pyridine as solvent transformation was, under the experimental conditions employed, not obtained with the following reagents:—aluminium alkoxides; calcium metal; calcium carbide; calcium hydroxide; magnesium metal; sodium bismuthate; sodium borate; sodium cyanide; hydroxypentamminocobaltic chloride The basic action of the latter salt involves the reaction:—

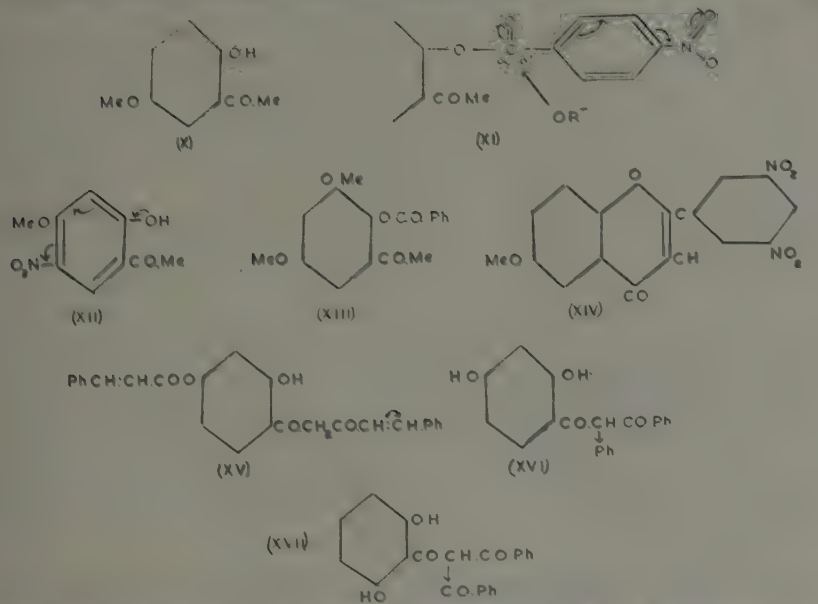
$$[Co(OH)(NH_3)_5]^{++} \longrightarrow [Co(H_2O)(NH_3)_5]^{+++}$$

Sodamide in piperidine or quinoline was also ineffective.

Taken as a whole, the results given above indicate that the transformation under discussion involves a base-catalysed intramolecular condensation of the Claisen type.

Transformation of Nitro-esters.

Support is provided for these views, by results which have been obtained with various nitrobenzoic esters of (1) and of 2-hydroxy-5-methoxy-acetophenone (X). The presence of the nitro-group in compounds of the type (XI) renders the C_α more electrophilic, thus facilitating not only transformation but also reaction with the anion of the transforming agent. With the 3:5-*dinitrobenzoic ester* of (I), sodium ethoxide gave ethyl 3:5-dinitrobenzoate, and sodamide, 3:5-dinitrobenza-



mide. Transformation of nitro-esters can, however, be effected with the sodium salts of (I) and (X), and with sodium hydroxide, sodium hydride, sodium peroxide, and ethyl sodioacetoacetate, though the peroxide produced violent reaction with the dinitro-esters. It should be mentioned that Ullal, Shah, and Wheeler, (22) found that sodium ethoxide in alcohol caused alcoholysis without transformation with esters of (XII) in which the nitro-group in the acetophenone nucleus renders the C_α (See XI) electrophilic.

Effect of methoxyl groups.

The tendency of the methoxyl group in esters of (X) to release electrons to the *p*-side chain, and so reduce the electrophilic nature of the ester carbonyl carbon, hinders transformation. Thus sodium methoxide did not transform the *benzoic ester* of (X), although with sodium peroxide a yield of 76% of diketone was obtained. Similarly Baker, Brown, and Scott, (3) were unable to transform 2-benzoyloxy-3:5-dimethoxyacetophenone (XIII) with sodamide in toluene. In the present research it was not found possible to transform the *β-phenylpropionic ester* of (I)—presumably the anionoid effect of the alkyl chain in the acid radical reduces the electrophilic action of the ester carbonyl carbon. On the other hand the *cinnamic and* m-*nitrocinnamic* esters of (I), in which the anionoid effect is reduced, were both readily transformed to the corresponding o-*hydroxydiketones*, which were cyclized respectively to 2-styrylchromone—an authentic sample of which was prepared from 2-methylchromone (28) and benzaldehyde, by the method of Cheema, Gulati and Venkataraman (8)—and 2-(m-*nitrostyryl*)*chromone*.

As a matter of interest attempts were made to transform the o-*toluene-sulphonic ester* of (I) but without success—further experiments with sulphonic esters are in hand.

Cyclization of 1:3-*Diketones.*

A variety of reagents have been used for this cyclization, of which three of the most useful, concentrated sulphuric acid, and acetic acid containing sodium acetate or a trace of mineral acid, are due to Wittig (27) and Baker (1). Hydrogen bromide in acetic acid is also satisfactory (21, 24). Pyridine with a trace of mineral acid has now been found efficient, but shows no advantage over the reagents previously employed.

Preparation of flavone. The facts that yields of about 80% can now be obtained in the transformation of *o*-benzoyloxyacetophenone into *o*-hydroxydibenzoylmethane makes it possible to prepare flavone from *o*-hydroxyacetophenone (I – V; R = Ph) with a yield of over 40%. Furthermore the sequence of operations can be carried through within two hours, so that the route thus provided for the preparation of flavone is, perhaps, more satisfactory than those hitherto available. For example, direct aroylation of (1) by treatment with benzoic anhydride and sodium benzoate for eight hours gives a yield of flavone of less than 10% (7), while selenium dioxide dehydrogenation during twelve hours of *o*-hydroxyphenylstyryl ketone ($C_6H_4(OH).CO.CH:CHPh$), which must first be prepared from *o*-hydroxyacetophenone, gives flavone in a 43% yield (16).

The Effect of Nitro-groups on Cyclization.

The presence of nitro-groups in the acid portion of an ester of *o*-hydroxyacetophenone facilitates cyclization of the related *o*-hydroxydibenzoylmethane. Thus, the 3:5-*dinitrobenzoic ester* of (X), when treated with the sodium salt of that phenol in pyridine gave on acidification 3′:5′-*dinitro*-6-*methoxyflavone* (XIV), and not the expected diketone. Again the diketone from the *m*-nitrobenzoate of (1) was cyclized by heating above the melting point. These and the facile cyclization of 3:5-*dinitro*-2′-*hydroxydibenzoylmethane* (Table 4) are possibly due to the *−I, −T,* effects of the nitro-group, which renders a methylene hydrogen of the 1:3-diketone more labile, thus promoting enolization and consequently cyclization. A survey of the literature confirms this view, since in general when the intermediate diketone cyclizes readily it contains an electrophilic group. Compounds (XV) and (XVI), (1) and (XVII) (2) provide examples of diketones which readily yield the corresponding chromones.

Nitroflavones. The preparation of simple nitroflavones was attempted by Bogert and Marcus (6), who by the direct nitration of flavone obtained a mixture of 2′-, 3′-, and 4′-nitroflavone, which could not be separated. The corresponding aminoflavones were, however, prepared from the mixed product in a pure state by reduction. 2′- and 4′-*Nitro-* and 3′:5′-*dinitroflavone* have now been synthesised by the transformation reaction. Virkar (23), had previously obtained 3′-nitroflavone. 3′:5′-*Diaminoflavone* and its *diacetyl derivative* have also been prepared.

EXPERIMENTAL

Preparation of o-Hydroxyacetophenones and their sodium salts.

o-Hydroxyacetophenone (1) (17) and 2-hydroxy-5-methoxyacetophenone (X) (3) were prepared according to the methods given by the authors cited. The sodium salts of these phenols separated when a molecular proportion of sodium methoxide

was shaken with a solution of the phenol in dry ether (29). The precipitated salts were dried under reduced pressure.

Preparation of acid chlorides.

The acid chlorides required for the preparation of the esters of *o*-hydroxyacetophenones (I and X) were, if not available, obtained by removing under reduced pressure excess of reagent from a solution of the acid in thionyl chloride, which had been heated under a reflux until the evolution of hydrogen chloride and sulphur dioxide ceased, moisture being excluded.

Preparation of o-aroyloxyacetophenones.

A solution of the phenol in pyridine (about 3 ml. of solvent per g.), which had been treated with the relevant acid chloride (1.1. mol.) and either heated at 100° for 15-30 min. or preferably kept at room temperature overnight, was poured into excess of aqueous mineral acid (up to 5%). The solid product, after it had been washed with aqueous sodium hydroxide (2%) until it gave no colour in alcoholic solution with aqueous ferric chloride, with aqueous acetic acid (10%), and with water, was recrystallised from a suitable solvent.

As o-(*β-phenylpropionyl*)*oxyacetophenone* did not solidify when the reaction mixture from (I) and the acid chloride were poured into dilute acid, an ethereal solution of the resulting oil was washed with aqueous sodium hydroxide (2%) and dried over anhydrous sodium sulphate. The liquid remaining after removal of the solvent solidified after distillation at 200-220°/1mm.

Of the esters prepared in the course of this work *o*-benzoyloxyacetophenone (ester serial No. 1) (1: see below under *Preparation of flavone from o-hydroxyacetophenone*) and *o*-(3-nitrobenzoyloxy)acetophenone (ester serial No. 3) (23) have already been described. The remainder, which are new, are given in Table 2.

TABLE 2.

Esters of *o*-hydroxyacetophenone (I) and of 2–hydroxy–5–methoxy–acetophenone (X).

Phenol	Acyl radical of ester.	Ester serial No.	m.p.	Crystallising solvent	Formula	Found %			Required %		
						C	H	N	C	H	N
I	2-Nitrobenzoyl-	2	123–5°	Alcohol	$C_{15}H_{11}O_5N$	63·3	4·1	4·7	63·2	3·9	4·9
I	4-Nitrobenzoyl-	4	91–2°	Aqueous alcohol	,,	63·3	3·9	5·1	,,	,,	,,
I	3:5-Dinitrobenzoyl-	5	131–3°	Alcohol-acetone	$C_{15}H_{10}O_7N_2$	54·0	3·4	9·0	54·6	3·0	8·5
I	β-Phenylpropionyl-	6	36°	Methyl alcohol–light petroleum (60–80°)	$C_{17}H_{16}O_3$	76·7	6·0	—	76·1	6·0	—
I	Cinnamoyl-	7	73°	Alcohol	$C_{17}H_{14}O_3$	76·1	5·4	—	76·7	5·3	—
I	3-Nitrocinnamoyl-	8	122–4°	Acetone and ligroin	$C_{17}H_{13}O_5N$	65·6	4·1	5·3	65·6	4·2	4·5
I	2-Toluenesulphonyl-	9	90–1°	Aqueous alcohol	$C_{15}H_{14}O_4S$	61·5	5·0	—	62·1	4·8	—
						S = 10·8			S = 11·0		
X	Benzoyl-	10	55–7°	Aqueous acetic acid and light petroleum (40–60°)	$C_{16}H_{14}O_4$	71·1	5·2	—	71·1	5·2	—
X	4-Nitrobenzoyl-	11	146–7°	Alcohol (charcoal)	$C_{16}H_{13}O_6N$	60·9	3·8	4·3	61·0	4·1	4·4
X	3:5-Dinitrobenzoyl-	12[1]	139–40°	Alcohol	$C_{16}H_{12}O_8N_2$	53·2	3·4	7·8	53·3	3·3	7·8
X	Cinnamoyl	13[1]	90°	Alcohol	$C_{18}H_{16}O_4$	72·5	5·2	—	73·0	5·4	—

[1] Compounds (12) and (13) formed yellow crystals, the remainder were colourless.

Action of heat on a pyridine solution of o-benzoyloxyacetophenone.

The solution (1 g. in 15 ml.) was heated at 275° in a sealed tube for 24 hours and the product was mixed with an excess of dilute hydrochloric acid. Crystals of flavone (m.p. 93-4° and mixed m.p. 95-6°, with an authentic specimen, m.p 97°) eventually formed on the surface of the tarry precipitate.

Preparation of 1:3-Diketones.

Transformation of o-benzoyloxyacetophenone into o-hydroxydibenzoylmethane. (Table 1).

Alkali metals. A solution of *o*-benzoyloxyacetophenone (m.p. 87°; 0.5 g.) in pyridine (*ca* 10 ml.) was treated with the alkali metal (1 atom) in small lots, and the mixture was heated under reflux until reaction ceased; the times required were: Li, 30 min.; Na, 15 min.; K and Rb, 7 min. The cooled product was acidified with dilute acetic acid (10%; caution, unchanged metal) and the precipitated *o*-hydroxydibenzoylmethane removed by filtration.

The procedure with acetone and dioxan was similar. Using the former solvent reaction was complete in 10 min. There was some interaction between potassium and acetone, but this did not interfere with the transformation. With rubidium, however, the vigorous reaction between the metal and the solvent caused inflammation. Reaction in dioxan as solvent was complete in 20 min.

Alkali metal carbonates. A slight excess (more than 1 mol.) of the carbonate was employed, and heating under a reflux continued for the times shown in Table 1. The product was separated by the addition of dilute acid.

Other transforming agents. Generally a solution of the ester (0.5 – 1 g.) in pyridine (10-20 ml.) or other solvent was heated with agitation at up to 115° for about 15 min. with the transforming agent (*ca.* 1.2 mols.), and the liquid acidified with dilute acetic acid (10%) in the cold to precipitate the diketone. Care in acidification is necessary with alkali hydroxides, sodium hydride, sodium peroxide, and sodamide. (See also below under *Preparation of flavone from o-hydroxyacetophenone.*) Modifications in the general method required with some of the reagents used are set out below:—

Triphenylmethylsodium. A solution of *o*-benzoyloxyacetophenone (0.5 g.) in anhydrous ether (10 ml.) contained in a reaction flask filled with nitrogen was treated with a solution of triphenylmethylsodium, prepared, stored, and delivered into the reaction flash in an atmosphere of nitrogen using the procedure of Hauser and Hudson (11). Addition of the reagent was stopped when the red colour of its ethereal solution was no longer discharged. The reaction mixture was acidified with aqueous acetic and (10%), and the ethereal layer extracted with aqueous sodium hydrogen carbonate (2%) to remove acid, and with aqueous potassium hydroxide (2%) until colourless. The extracted diketone was precipitated from the alkaline solution by the addition of dilute acid and recrystallised from alcohol.

Sodamide in liquid ammonia. Liquid ammonia was added from a cylinder to *o*-benzoyloxyacetophenone (1 g.) and an excess of sodamide in a Dewar flask, and the mixture was kept for 48 hours, by which time the ammonia had evaporated. The residue was treated with water (caution) and the solution acidified with dilute acetic acid to precipitate a brown tar, which on recrystallisation from alcohol yielded *o*-hydroxydibenzoylmethane.

Grignard reagents. A solution of *o*-benzoyloxyacetophenone (1 g.) in anhydrous ether (12 ml.) was added to anhydrous ether (5 ml.) containing a Grignard reagent prepared from magnesium ribbon (0.1 g.) and bromobenzene (0.7 g.), and the mixture was heated under a reflux for 5 min. and acidified with dilute hydrochloric acid. The diketone was recovered from the ethereal layer as with triphenylmethylsodium. A similar reaction was carried out with a Grignard reagent prepared from methyl iodide.

Sodium aluminate. A solution of *o*-benzoyloxyacetophenene (0.5 g.) in pyridine (15 ml.) containing an excess of sodium aluminate was heated under a reflux for 10 min., and cautiously acidified with sulphuric acid (50%). The supernatant diketone was, as far as possible, separated mechanically, and the residual liquid and solid were extracted with ether to recover a further quantiy of diketone.

Potassium benzoate and potassium acetate. With these salts heating under reflux was continued for eight hours. Using potassium acetate the mixture was acidified to precipitate the diketone. With potassium benzoate the precipitate obtained on acidification was dissolved in ether, and the solution extracted with aqueous sodium hydrogen carbonate to remove benzoic acid. The solvent was then volatilized.

Preparation of 1:3-*Diketones other than* o-*Hydroxydibenzoylmethane.*

Table 3 gives particulars of the transformation of a number of esters of *o*-hydroxyacetophenones using either the method given above under *Other transforming agents,* (Method A) or a modification, in which the solution of the ester in pyridine was kept in contact with the reagent at room temperature for 24-48 hours (Method B). It was not found possible to transform either *o*-(β-propionyloxy)acetophenone (ester serial No. 6) or *o*-toluenesulphonyloxyacetophenone (ester serial No. 9). 2-(3':5'-Dinitrobenzoyloxy)-6-methoxyacetophenone (ester serial No. 12) yielded the corresponding *flavone* directly on transformation.

TABLE 3.

Hydroxy-1:3-diketones prepared by the Transformation of the corresponding Esters.

Ester serial No.	1 : 3–Diketone	Diketone serial No.	m.p. and solvent	Formula and description	Elements	Found %	Required %	Method used, transforming agent[1] and crude yields
2	2-Nitro-2′-hydroxy-dibenzoylmethane	1	162–3° Dioxan	$C_{15}H_{11}O_5N$ Yellow crystals	C H N	63·3 4·1 4·9	63·2 3·9 4·9	B. NaOH, 36%; NaH, 80%; NaA, 50%; Na_2O_2, 60%.
3	3-Nitro-2′hydroxy-dibenzoylmethane(23)	2	154–7° Alcohol-acetone and aq. acetic acid	—	—	—	—	B. NaAr, 60%.
4	4-Nitro-2′-hydroxy-dibenzoylmethane	3	198–201° Benzene	$C_{15}H_{11}O_5N$ Yellow crystals	C H	63·4 4·0	63·2 3·9	A. NaA, 47%.
5	3 : 5-Dinitro-2′-hydrozy-dibenzoylmethane	4	227–8°	$C_{15}H_{10}O_7N_2$ Golden brown plates	C H N	55·1 3·4 8·0	54·6 3·0 8·5	B. NaAr, 70%; NaH & NaA also effective[2]
7	2-Hydroxybenzoyl-cinnamoylmethane	5	134° Alcohol	$C_{17}H_{14}O_3$ Pale yellow crystals	C H	77·1 5·0	76·7 5·3	A. NaOEt in alcohol (no pyridine), 25%.
8	2-Hydroxybenzoyl-3′-nitrocinnamoylmethane	6	158°–60° Benzene and glacial acetic acid	$C_{17}H_{13}O_5N$ Yellow rectangular crystals	C H N	64·9 4·4 4·9	65·6 4·2 4·5	B. NaAr, 70%.
10	2-Hydroxy-5-methoxy-dibenzoylmethane	7	81° Aq. alcohol	$C_{16}H_{14}O_4$ Bright yellow needles	C H	70·7 5·4	71·1 5·2	B. Na_2O_2, 76%; NaOMe did not effect transformation.
11	4-Nitro-2′-hydroxy-5′-methoxydibenzoyl-methane	8	173–4° Alcohol	$C_{16}H_{13}O_6N$ Yellow crystals	C H N	60·2 4·4 4·1	61·0 4·1 4·4	B. NaA, 30%; NaOMe, 12% (alcoholysis).
13	2-Hydroxy-5-methoxy-benzoylcinnamoyl-methane	9	95–7° Aq. alcohol	$C_{18}H_{16}O_4$ Dark yellow crystals	C H	72·6 5·2	73·0 5·4	A, NaOMe, 60%.

[1] NaA = Ethyl sodioacetoacetate; NaAr = Na salt of *o*-hydroxyacetophenone.

[2] When the transformation of 3:5-dinitrobenzoyloxyacetophenone was attempted using sodium ethoxide in pyridine, alcoholysis occurred, and ethyl 3:5 dinitrobenzoate, m.p. 90-1° (lit. 93-4°), (Found: C, 44.8; H, 3.3; Calculated for $C_9H_8O_6N_2$, C, 45.0; H, 3.3%) was obtained. The use of sodium peroxide caused a violent reaction, while triphenylmethylsodium in chloroform or pyridine gave no useful result. 3:5-Dinitrobenzamide m.p. 183° (lit. 183°) was obtained, when sodamide in pyridine was employed.

Cyclization of Hydroxy-1:3-diketones.

In general a solution of the diketone in glacial acetic acid containing a few drops of mineral acid was heated to boiling, cooled, and diluted with water to precipitate the chromone. Baker (1), obtained quantitative yields of flavone in the

cyclization of *o*-hydroxydibenzoylmethane with concentrated sulphuric acid in the cold, or with glacial acetic acid containing sodium acetate at the boiling point for 30 min.

Table 4 gives particulars of the chromones, mainly flavones, prepared in the course of the present work.

TABLE 4.

Chromones prepared by Cyclization of Hydroxy–1 : 3–diketones.

Diketone serial No.	Chromone	m.p. and crystallizing solvent	Formula and description	Elements	Found %	Required %	Yield %	Method of cyclization[1]
1	2′-Nitroflavone	180–2° Dioxan	$C_{15}H_9O_4N$ White prisms	C H N	67·3 3·4 5·2	67·4 3·4 5·2	50	a
2	3′-Nitroflavone(23)	201–3° Dioxan	—	—	—	—	—	b
3	4′-Nitroflavone	244–6° Glacial acetic acid and acetone-dioxan	$C_{15}H_9O_4N$ Colourless crystals	C H N	67·0 3·6 4·5	67·4 3·4 5·2	80	a
4	3′ : 5′-Dinitroflavone	297–9° Acetic acid and dioxan	$C_{15}H_8O_6N_2$ Colourless crystals	C H N	57·4 2·7 9·2	57·7 2·6 9·0	—	a, c
5	2-Styrylchromone[2]	138–40° Alcohol	—	—	—	—	—	a
6	2-(*m*-Nitrostyryl)-chromone	233–5° Dioxan	$C_{17}H_{11}O_4N$ Pale yellow crystals	C H N	69·3 3·7 5·2	69·6 3·8 4·8	56	a
7	6-Methoxyflavone(12)	162–3° Aqueous alcohol	—	—	—	—	70	a
8	4′-Nitro-6-methoxy-flavone	205–6° Dioxan-water	$C_{16}H_{11}O_5N.H_2O$ Pale yellow crystals	C H N H_2O[3]	61·4 4·0 4·5 5·5	61·0 4·1 4·4 5·7	60	a
9	6-Methoxy-2-styryl-chromone	145–6° Aqueous alcohol and ligroin	$C_{18}H_{14}O_3$ Colourless needles	C H	77·1 5·2	77·7 5·0	80	a

[1] Methods of cyclization:—
(a) Glacial acetic acid with a trace of mineral acid at 100°
(b) Heating above m.p.
(c) The diketone m.p. 227-8°, was heated in a silicone bath (b.p. 300°), when formation of the flavone (m.p. 296-300° from dioxan), occurred with solidification.

[2] Cheema, Gulati and Venkataraman (8) give m.p. 131°

[3] Loss in weight at 100° when heated under reduced pressure for one hour.

Preparation of flavone from o-hydroxyacetophenone.

A mixture of *o*-hydroxyacetophenone (6.8 g.), benzoyl chloride (7.0 g.) and pyridine (10 ml.), which had been heated (calcium chloride guard tube) at 100° for 15 min. (See (1)) was poured into dilute hydrochloric acid (1%; 300 ml.), and the solid product was washed and sucked at the pump for 5 min. The crude ester was dissolved in pyridine (30 ml.), and the solution treated at 50° with potassium hydroxide (2.5 g.), which had been pulverized in a hot mortar. The mixture was agitated for 5 min., during which time a copious precipitate of the yellow potassium salt of *o*-hydroxydibenzoylmethane was formed. Excess of aqueous acetic acid (10%) was then added, and the precipitated diketone was freed from adhering liquid at the pump. It was dissolved in concentrated sulphuric acid (70 ml.) and after 15 min. the acid solution was poured into crushed ice. The precipitated flavone (4.7 g.: 42% yield) had m.p. 92–3°; mixed m.p. with an authentic specimen (m.p. 97°), 93–5°. The yield of crude ester from *o*-hydroxyacetophenone was 70%, of crude diketone from crude ester 78%, and of flavone from crude diketone 77%.

Preparation of 3′:5′-Dinitro-6-methoxyflavone (XIV) *from 2-(3′:5′-dinitrobenzoyloxy)-5-methoxyacetophenone.*

The sodium salt of 2-hydroxy-5-methoxyacetophenone (X; 2.5 g.) was added to a solution of 2-(3′:5′-dinitrobenzoyloxy)-5-methoxyacetophenone (4 g.) in pyridine (20 ml.), and after it had been kept for three days, the mixture was acidified with dilute hydrochloric acid (10%). The brown solid thus obtained—the chloroform solution of which was not extracted with aqueous sodium hydroxide (2%) and was, therefore, not the diketone—separated from glacial acetic acid in pale yellow needles, (2 g.) m.p. 301–3°, which gave a pale green fluorescence with sulphuric acid. (Found; C, 56.4; H, 3.3; N, 7.9. $C_{16}H_{10}O_7N_2$ requires C, 56.1; H, 2.9; N, 8.2%).

Preparation of 3′:5′-Diaminoflavone.

A suspension of 3′:5′-dinitroflavone (2.5 g.) in hot concentrated hydrochloric acid (45 ml.) was treated with stannous chloride (11 g.), and the white precipitate, which replaced the origional flavone, was separated after the mixture had been kept at 0° C. for some time, washed with concentrated hydrochloric acid and dissolved in water. The resulting solution was made strongly alkaline with aqueous sodium hydroxide (20%), and the free *base* thus obtained crystallised from alcohol, from which it separated in yellow crystals having a m.p. 237°. Yield, 0.5 g. Found: :C, 71.4; H, 4.9; N, 10.8. $C_{15}H_{12}O_2N_2$ requires C, 71.3; H, 4.8; N, 11.1%).

The *diacetyl* derivative (pyridine-acetic anhydride) had m.p. above 350° on recrystallisation from glacial acetic acid. (Found C, 67.9; H, 4.8; N, 8.3 $C_{19}H_{16}O_4N_2$ requires C, 67.9; N, 4.8; N, 8.3%).

Summary

A variety of salts of weak acids, *e.g.* sodium hydroxide, phenoxide, peroxide, hydride, ethyl sodioacetoacetate, triphenylmethylsodium, potassium acetate and benzoate have been found to be effective reagents for the transformation of *o*-aroyloxyacetoarones into the corresponding *o*-hydroxydiaroylmethanes, which can be

cyclized to the corresponding chromones. The results obtained indicate that the reaction inolves a base-catalysed intramolecular condensation of the Claisen type. The effect of nitro- and methoxyl groups on the transformation is discussed, and the preparation of some new flavones is described.

The thanks of the authors are due to Professor Joseph Algar and Dr. Vincent Barry for advice.

Analyses by Drs. Weiler and Strauss (Oxford).

DEPARTMENT OF CHEMISTRY,
UNIVERSITY COLLEGE,
DUBLIN.

REFERENCES.

(1) Baker, *J. C. S.*, 1933, 1381.
(2) Baker, *J.C.S.*, 1934, 1953.
(3) Baker, Brown and Scott, *J.C.S.*, 1939, 1922.
(4) Bell, *Acid-Base Catalysis*, (Oxford, 1941), 41.
(5) Bhalla, Mahal and Venkataraman, *J.C.S.*, 1935, 868.
(6) Bogart and Marcus, *J. Amer. Chem. Soc.*, 1919, **41**, 83.
(7) Chadha and Venkatarman, *J.C.S.*, 1933, 1073.
(8) Cheema, Gulati and Venkataraman, *J.C.S.*, 1932, 925.
(9) Hann and Lapworth, *J.C.S.*, 1904, **85**, 46.
(10) Hansley and Carlisle, *Chem. Eng. News.*, 1945, **23**, 1332.
(11) Hauser and Hudson, *Organic Reactions*, **1**, (Wiley, New York, 1942), 266.
(12) Hill and Melhuish, *J.C.S.*, 1935, 1161.
(13) Lapworth, *J. C. S.*, 1901, **79**, 1265.
(14) Mahal and Venkataraman, *Current Science*, 1933, **2**, 214.
(15) Mahal and Venkataraman, *J.C.S.*, 1934, 1767.
(16) Mahal, Rai and Venkataraman, *J.C.S.*, 1935, 866.
(17) Mozingo, *Organic Syntheses*, **21**, (Wiley, New York, 1941), 45.
(18) Pauling, *Nature of the Chemical Bond.* (Cornell University Press, New York, 1939), 64.
(19) Swamer and Hauser, *J. Amer. Chem. Soc.*, 1946, **68**, 2647.
(20) Ullal and Wheeler, *Current Science*, 1938, **7**, 280.
(21) Ullal, Shah and Wheeler, *J.C.S.*, 1940, 1499.
(22) Ullal, Shah and Wheeler, *J. Univ. Bombay*, 1941, **10**, 118.
(23) Virkar, *J. Univ. Bombay*, 1942, **11**, 136.
(24) Virkar and Wheeler, *J.C.S.*, 1939, 1679.
(25) Watson, *Ann. Repts. Chem. Soc.*, 1941, **38**, 115.
(26) Watson, *Trans. Faraday Soc.*, 1941, **37**, 707.
(27) Wittig, *Annalen*, 1926, **446**, 155.
(28) Wittig, *Annalen*, 1926, **446**, 170.
(29) Wittig, *Annalen*, 1926, **446**, 199.

No. 27.

SEED TRANSMISSION OF VIRUS YELLOWS OF SUGAR BEET (*Beta vulgaris* L.) AND THE EXISTENCE OF STRAINS OF THIS VIRUS IN ÉIRE.

By

Phyllis E. M. Clinch and J. B. Loughnane.
(Department of Plant Pathology, University College, Dublin).

(Plates 13–17).

(Read February 24. Published Separately July 29, 1948).

Introduction.

Sugar beet is a comparatively new crop in Éire and it was not until recent years that abnormal yellowing of the beet foliage became a matter of grave concern to the Irish Sugar Company's Agricultural Advisers. From the descriptions of Dutch and English workers (11) (15) (8) it was apparent to them that much of this yellowing was probably of virus nature, corresponding with the disease known as " vergelingsziekte " in Holland, " jaunisse " in Belgium, and " virus yellows " in England. However, the situation was complicated by the existence of other factors liable to induce yellowing in beet, such as Downy Mildew (*Perenospora Schachtii*) and certain mineral deficiencies in the soil. Yellowing was particularly widespread in 1946 and in August of that year it was arranged that affected plants should be examined in this laboratory in order to identify with certainty diseases of virus origin. Dr. M. A. Watson, of Rothamstead Experimental Station, visited Eire in October, 1946, and expressed the opinion that most of the yellowing present in the field crops (100% in certain cases), was due to virus infection.

Particular interest was centred on a yellowing which occurred in a new variety (family 41) of sugar beet bred by Mr. B. Crombie at the Sugar Company's Station at Mallow, Co. Cork. The cross had been made in a pollen-proof cage in 1945, the parent plants having been selected from commercial crops. In 1946 three separate crops from the resulting seed showed, a few weeks after singling, approximately 25% of yellowing which, according to Mr. Crombie, resembled typical virus yellows, but did not appear to spread to neighbouring beet crops. The manner of its occurrence suggested that this form of yellows was seed-borne. However, Quanjer (11) who had studied virus yellows for a considerable period, found no evidence that the disease was transmitted through the seed ; and although published accounts of tests with seed from diseased plants are scarce, it appears to be generally accepted that virus yellows is not seed-borne. It was, therefore, tentatively assumed that the

yellowing in family 41 was probably not of virus nature, but was a variegation resulting from a genetical abnormality. Transmission tests in this laboratory subsequently proved this assumption to be incorrect, and a note to this effect has already been published (5). A more detailed account of the experiments with family 41 and with specimens from commercial beet crops is now submitted.

Materials and Methods.

Healthy and affected field-grown plants of family 41, together with a small quantity of the residual seed of that family, were received from the Sugar Company on 7th September, 1946. For the testing of commercial crops specimens of sugar beet and mangold plants showing virus-like symptoms of the type described by Hull and Watson (8) were selected from field crops in Dublin and Carlow and re-planted in pots in a glasshouse at the Albert Agricultural College, Glasnevin. The specimens were full-grown plants all of which showed the typical thickening and yellowing of the lower leaves. A small number of the sugar beet plants showed, in addition, mottling and more or less crinkling of the leaves and were selected as possible examples of mixed infections with the yellows and mosaic viruses.

The virus content of each specimen was determined by carrying out transmission tests from the affected plants to healthy plants, using as vectors the aphides *Myzus persicae* (Sulz.) and *Aphis Fabae*, both of which were shown by Roland (11) and van Schreven (13) to transmit yellows and mosaic viruses of sugar beet. Sap inoculations using carborundum powder were also performed with a view to separating the mosaic virus, if present, from yellows. Sugar beet of a commercial variety (No. 347) obtained from Mr. Crombie, and spinach (*Spinacia oleracea* L.), were used as experimental hosts. The seed was usually sown in 10-inch pots and thinned to 7–10 seedlings which were submitted to infection when the first pair of true leaves were about 3 inches in length. Various larger sizes were also experimented with, including well-developed plants each in a 7-inch pot. Susceptibility did not appear to vary to any appreciable extent with size, provided the plants were in an active state of growth during the experimental period.

The stock of *Myzus persicae* used in the experiments was reared on turnip and cabbage plants ; *Aphis Fabae* was collected from the spindle tree (*Euonymus*), and later reared on beans. The transmission tests were carried out in a glasshouse in insect-proof cages constructed of timber with two sides of very fine mesh copper gauze, the top and remaining sides being of glass. The bottom of each cage consisted of perforated zinc which was covered by two 3-inch layers of gravel and fine sand, respectively. The plant to be tested was placed in a cage and colonised with the appropriate vector. When the insects had multiplied sufficiently, the pots containing the experimental seedlings were included in the cage and allowed to remain for 2–7 days. The longer period was allowed in the earlier experiments in order to ensure considerable movement of insects from the source to the healthy plants. At the end of the feeding period the plants were fumigated with nicotine vapour and moved to another glasshouse for observation. When necessary the seedlings in 10-inch

pots were later transplanted separately to 7-inch pots thus permitting an extended observation period. Transplants were also made to outdoor plots in order to observe symptoms under field conditions.

INVESTIGATION OF YELLOWING IN FAMILY 41.

A small portion of the seed of this family was sown in September 1946 and the remainder on 10th March, 1947, both lots being kept in separate insect-proof glasshouses. The Autumn sown seedlings were transplanted to 7-inch pots when of appropriate size and kept over Winter in an unheated glasshouse. During the dormant period they already had 2–4 true leaves approximately 5 inches in length, but these showed no signs of virus infection. Owing to severe weather active growth was not resumed until the last week of March, 1947. On 22nd April, the first signs of yellowing were observed at the tips of the lower leaves of 2 plants. During the following 3 weeks further plants of the batch showed similar symptoms. In all, 11 out of 33 plants were affected. The seedlings from the March sown seed received no check in growth. They were transplanted to 7-inch pots when the first pair of true leaves were about 3 inches in length and proceeded to grow vigorously. Only one plant appeared stunted and by 16th May, the two basal leaves of this plant were distinctly yellow at the tips, had ceased to expand and were tough and leathery in texture. All the remaining plants appeared healthy until they were about 10 inches in height with 10–12 foliage leaves. Then the tips of the lower leaves of some of them began to thicken and turn pale, eventually becoming yellow or orange in colour. Between May 17th and June 7th, 57 of the 110 plants developed pathological symptoms. Where two or three plants derived from an individual cluster, there was no uniformity in the number showing yellowing. One or both of a pair or two out of three might be affected ; on the other hand a single cluster might give rise only to healthy plants. However, the majority of the seedlings raised were from separate clusters.

The yellowing usually spread uniformly from the tips to the proximal ends of the leaves, only the areas around the veins and the basal portion at the junction of lamina and petiole remaining green (Fig. 1.). Sometimes the spread was asymmetrical, one side of a leaf being more extensively affected than the other (Fig. 3.). Occasional leaves showed yellowing at the tip (Fig. 2) sometimes accompanied by isolated patches of affected tissue elsewhere on the lamina which frequently were situated at the junction of two main veins. Thickening of any portion of the leaf was inevitably followed by yellowing. There was no discernible abnormality in the non-yellowed areas of affected leaves. Eventually the chlorotic areas withered and turned brown and the margins of the leaves curled upwards (Fig. 4). Various saprophytic moulds developed on the dead areas. Taking into account the number of infections in the Autumn sowing, the percentage of infected plants from the 143 seed clusters sown was 47.5.

The symptoms described above are essentially similar to those of virus yellows of sugar beet as described by Quanjer (10) Roland (11), and Watson (15). Demarcation of yellow from green areas by the veins, as seen in family 41, was described

in the case of virus yellows by Klinkenberg (9). Watson (15) (Plate 12, Fig. 4) and Hale, Watson and Hull (7) (Plate 1, Fig. 5) illustrate this condition in leaves from field plants, but considered this localization to be the result of late infection of well developed plants.

Transmission Tests with plants of Family 41 showing Yellowing.

It has already been reported (5) that transmission of the yellows disease in family 41 to healthy sugar beet and spinach seedlings was obtained in three different experiments using *Myzus persicae* as vector. Of the 68 sugar beet and 34 spinach plants submitted to infection, 85% and 76%, respectively, developed yellowing. In three further experiments performed during August and September, 1947 and using different plants of family 41 as sources, similar results were obtained. No infection was secured in three experiments carried out during June, July and August, using *Aphis Fabae* as vector and involving 48 sugar beet seedlings as experimental hosts. In parallel experiments with mild virus yellows, *A. Fabae* transmitted the disease to only 2 of the 48 experimental plants.

The symptoms in the experimental plants began to appear 3–4 weeks after infection when the tips of the lower leaves became tough and leathery and then gradually turned yellow. There was no vein clearing and the ensuing disease was essentially similar to a mild virus yellows isolated from commercial crops which is described in another section of this paper. The affected plants were only slightly stunted compared with healthy ones. As the symptoms were more or less confined to the lower leaves, they showed up best under conditions favouring the growth of sugar beet which in the glasshouse at Glasnevin are those prevailing during the Spring and Autumn months. Hot, sunny weather was unsuitable for the display of symptoms under glass as the plants tended to wilt and the lower leaves to wither. On the other hand, symptoms of yellowing were repressed if the plants were grown in a very dull light, due presumably to the lowered photosynthetic activity and consequent reduction in the level of surplus carbohydrate. This is true of mild virus yellows isolated from field crops as well as of the disease transmitted from family 41. Plants grown out-of-doors showed consistently good symptoms during the season.

The foregoing results proved conclusively that the yellowing in family 41 was due to a seed-borne virus, and it is assumed that one, or both, of the parents of this family was infected with virus yellows. As seed transmission of this virus has not hitherto been observed, it must be concluded that virus yellows does not normally infect the embryo of the sugar beet seed. Indeed there is no information as to whether it occurs in any part of the seed. Bennett and Esau (2) have shown that the Curly Top virus of sugar beet reaches a very high concentration in the mature seed, but that the actual embryo appears never to become infected. The fact that virus yellows was apparently able to penetrate with ease into the embryo in the case of family 41 suggests that a genetical abnormality in this family may possibly have affected the permeability of certain cells of the embryo thus permitting diffusion of virus into regions where it does not normally penetrate. The behaviour of the seed in subsequent generations will probably throw some light on this subject.

Accumulation of Starch in Foliage of Family 41.

Quanjer (10) showed that starch accumulated in the yellowed leaves of virus-infected sugar beet plants and both Quanjer and van Riemsdijk (12) concluded that this resulted from inhibited translocation rather than from a disturbance in the respiratory mechanism. Tests for starch accumulation in affected leaves of family 41 were made by removing potted plants from the greenhouse at 5 p.m. on days of bright sunshine and placing them in a dark room for periods varying from 48 to 72 hours, after which appropriate leaves were cut from the plants, dipped in boiling water and decolorised by soaking in warm alcohol. They were then stained in a dilute solution of iodine. The information derived from several such tests performed during the months of April, May and June was as follows : After 48–72 hours in darkness no starch is demonstrable in the leaves of healthy plants nor in the leaves of affected plants which are of normal colour and texture. Starch accumulation is obvious in the toughened, but still green, areas of affected leaves, but is most conspicuous in the yellowed areas. The demarcation of affected and normal areas by the main veins in some leaves is striking (Figs. 5 and 6). Isolated yellow spots appear as dark blue patches on the decolorised and stained leaves. Examination of the lower leaves of old plants showed that prior to the onset of necrosis the starch begins to disappear from the yellow areas, being probably used in respiration. The regions which are first to become yellow are also those from which the accumulated starch is first to disappear for when old leaves showing general yellowing were stained with iodine after a period in darkness, only the interveinal areas of the distal portion of the leaf failed to turn blue. Staining of leaves of similar age showing yellowing only in the distal half, also revealed the disappearance of starch from the strongly chlorotic tip and accumulation of starch in the remainder of the yellow area. It was noteworthy in the case of these mature leaves that the green areas of those showing only partial yellowing were functioning normally in regard to translocation, being devoid of starch following a sojourn in darkness.

Hand sections of affected leaves of family 41 cut during the afternoon on days of bright sunshine showed the chloroplasts of the soft, green areas to be normal in appearance and indistinguishable from those of healthy leaves. In areas which were still green, but which had developed the toughened texture which precedes yellowing, the chloroplasts were gorged with starch, and this was also the situation in the yellow areas in the earlier stages. In the completely yellow or whitish areas of leaves affected for some time only the remains of degenerated chloroplasts were present. These are similar to the effects observed by Klinkenberg (9) in sections of leaves affected by virus yellows.

INVESTIGATION OF YELLOWING IN SUGAR BEET AND MANGOLD PLANTS SELECTED FROM FIELD CROPS.

All the plants selected from commercial crops as probable sources of virus were found to be infected, and a high percentage of infection in the experimental hosts was readily secured using *Myzus persicae* as vector. Repeated sap inoculations

from the various sources, however, with and without the use of carborundum powder, failed to produce any disease symptoms.

In all cases, including those in which the sugar beet sources had shown more or less crinkling and mottling, the disease transmitted by the insect vector was of the yellows type. There was no evidence of the presence of a mosaic virus in the specimens examined, and the mottling observed in some of them at pulling time was obviously not the result of virus infection. Two types of yellows disease were, however, isolated, which are believed to be strains of the same virus and which for convenience may be designated as mild yellows and etch yellows, respectively.

Mild Yellows.—The first symptoms of infection in rapidly growing seedlings of sugar beet and spinach appear about 18–21 days after inoculation and consist in a toughening of the tips of the lower pair of leaves which then lighten in colour and gradually turn yellow. The yellowing usually spreads downwards through these leaves, only the areas around the veins remaining green, and the texture of the leaves becomes thickened. Similar effects meanwhile appear in the next leaves above, so that the symptoms develop acropetally but do not advance beyond the medium sized leaves and are frequently confined to the lower ones. Later, the chlorotic areas of the older affected leaves become brown and necrotic and the entire lamina becomes dry and brittle and tends to curl upwards at the margins, especially in glasshouse plants. Growth of the infected plants is somewhat stunted compared with that of healthy plants.

Etch Yellows.—Under optimum conditions symptoms develop in sugar beet and spinach 12–14 days after infection. The veins of the young leaves appear translucent when viewed by transmitted light and later may become whitish or necrotic and appear as if " etched " on the leaf (Figs. 7 and 8). The vein clearing appears first at the tip of the leaf, then all over the lamina ; or it may spread asymetrically. About 16–21 days after infestation symptoms of yellowing develop independently of the etching in the lower leaves of the plant, in exactly the same manner as described for mild yellows. The yellowing proceeds acropetally but more leaves on the plant become involved than in the case of mild yellows and the plant as a whole is much more stunted. The etching of the veins is a primary symptom and is not apparent in young leaves which develop subsequently. It may, however, appear in the heart leaves of plants which have overwintered in the glasshouse when these resume growth in Spring. As the " etched " leaves increase in size they also turn yellow and rarely attain normal dimensions. In the later stages they may appear extremely tough and leathery, with sunken collapsed veins. A common feature of the etch-infected plants, especially under glasshouse conditions, is the development of minute reddish rings or spots on some of the older yellowed leaves (Fig. 9). This symptom did not appear to be associated with mild yellows and is possibly a characteristic, if not a constant symptom of the etch strain. However, further observation of plants affected with both strains, and grown under varying environmental conditions, would be necessary in order to confirm this view.

As already mentioned, the mild and etch yellows are both freely transmissible by *Myzus persicae*. Etch yellows is the more infectious of the two and the rate of

infection with this strain was usually 100%. *Aphis Fabae* proved to be a poor and erratic vector, and in four different experiments in which this aphis was employed to transmit mild yellows, the respective numbers of sugar beet seedlings which became infected were 3/24, 4/36, 0/24, 0/12. A somewhat higher rate of infection was obtained in the case of etch yellows. Both strains can be transmitted by *Macrosiphum solanifolii*, but the actual efficiency of this aphis as a vector of yellows has not yet been determined.

It was considered desirable to test the possibility that the veinal etching in "etch yellows" might be due to a contaminating virus in the original source of inoculum and not symptomatic of virus yellows. Accordingly, *Myzus persicae* was fed on (*a*) young leaves from the tops of sugar beet plants immediately after the development in them of vein clearing, and (*b*) lower leaves showing yellowing only. After 48 hours the insects were transferred to healthy sugar beet and spinach seedlings. In two such experiments it was found that vein clearing and subsequent yellowing developed in the test plants irrespective of whether the vectors had fed on sources (*a*) or (*b*). On the other hand, sap inoculations from similar leaves to healthy sugar beet and spinach seedlings failed to cause infection. Although these results do not eliminate the possibility that a mixture of viruses may be involved, they strongly support the view that the etching is merely a symptom of a particular strain of virus yellows and not that of a distinct virus.

The probable existence of strains of virus yellows was first suggested by Watson (14) on finding that the symptoms of the diseases which she isolated by insect from affected sugar beet specimens from Holland and from different counties in England differed in the intensity of their symptoms. The more severe strains were said to produce a vein clearing of the medium aged leaves (never the very youngest leaves), followed later by the appearance of local symptoms on the older leaves. No further references to the existence of strains of virus yellows appear to have been made, and in a recent paper by Hale, Watson and Hull (7) "clearing" and "etching" of the veins was described as a symptom of virus yellows associated with early infection of field plants. It was further stated by these authors that as the "etched" leaves develop they become yellowed so that in such plants the typical "yellows" symptoms occur first on leaves of medium age. In the present work it was found that the onset of yellowing occurs independently of the etching and that the latter is a primary symptom of what is apparently a particular strain of virus yellows and not simply the resultant of infection at a certain stage of development of the plant. The vein clearing appeared consistently, irrespective of whether the hosts were very young seedlings or well developed plants at the time of infection. It is also a persistent character having appeared in successive transfers from September 1946 to the end of the 1947 season.

Introduction of Etch Yellows to plants already infected with Mild Yellows.

Six plants of family 41, three infected with mild yellows and three apparently healthy, were colonised with viruliferous aphides (*M. persicae*) from a sugar beet plant infected with etch yellows. In due course all six plants developed vein clearing

in the young leaves showing that entry of the etch virus had not been prevented by the previous presence of the mild yellows. Following this a similar test was made using plants of the commercial variety No. 347 infected with the mild strain of yellows which had been isolated from various field plants. The result was the same as in the case of family 41, the experimental plants showing etching in the young leaves about 14 days after infection.

It has been established in the case of various sap-transmissible viruses, such as tobacco mosaic and potato virus X, that a plant infected with one strain cannot be infected by sap inoculation with another strain of the same virus. The evidence in regard to this cross immunity between strains has been summarised by Bawden (1). It indicates clearly that the " protection " is not due to inactivation of the one strain by the other but seems to depend entirely upon the inability of one strain to multiply in cells already occupied by another strain of the same virus. In the case of potato virus X, for example, it has been demonstrated that the protection is absolute in the parenchymatous tissues of the leaves and superficial layers of the stem of the potato. In such tissues the virus is believed to be generally distributed and present in high concentration. Complete protection, however, is obviously not afforded to the internal tissues of the stem for a second strain of X can be introduced to a plant already infected with this virus by means of a graft to either the aerial stem or tuber (3) (4). This is not due to absence of the " protecting " virus from the phloem, for it is known that virus X moves through the phloem although it may not multiply therein (3). It might be due to a lower concentration of virus in stem tissues. The authors are not aware of any experiments dealing with cross immunity between strains of insect-transmissible viruses in which the second strain is introduced (usually direct to the phloem) by means of the insect vector. The evidence in the case of virus X suggests strongly that there might not be any barrier to the entry of the second virus in these circumstances. Consequently, the ability of the etch virus to infect plants already containing a mild strain of virus yellows must not be taken as proof that the former is unrelated to the latter.

Internal Pathological Changes induced by Etch Yellows.

Quanjer (10) in 1934 was the first to draw attention to internal pathological changes associated with the yellows disease of sugar beet. He observed that in the secondary phloem of the yellowing leaves certain groups of sieve tubes and companion cells became filled with a yellow " gum " occupying the entire lumina of the cells, the walls of which also become yellow and sometimes swell. He referred to this condition as " gummosis " and contrasted it with the effects of the Curly Top disease of sugar beet in which hypertrophy and hyperplasia occur in the pericycle and phloem followed by the exudation from these cells of a viscid liquid as well as by extensive necrosis. From Quanjer's description of the symptoms it would appear that the strain of yellows with which he worked was of the mild type.

Watson (14) in 1940 reported failure to find gummosis in the phloem of glasshouse plants, even in very well-established infections. Klinkenberg (9), however, confirmed that gummosis is a constant feature of virus yellows but stated that it

develops only after the flow of carbohydrates has been arrested and should therefore be regarded as a secondary effect of the pathological condition of the phloem, rather than as the primary cause of inhibited translocation. She suggests, however, that there is probably a disturbance in the phloem prior to the appearance of its visible evidence in the form of gummosis.

Examination of leaves from sugar beet affected with etch yellows affords evidence that there is a direct effect on the phloem following infection with this virus. Sections were prepared from (*a*) a young tip leaf approximately 3 cms. in length and 1 cm. in width, 4 days after the development in it of vein-clearing and previous to the appearance of yellowing in the lower leaves of the plant, and (*b*) an upper leaf showing vein-clearing from a plant also showing yellowing in the lower leaves. The material was fixed in Regaud's fluid and embedded in paraffin. Sections were cut at 8μ and stained in Heidenhain's iron alum haematoxylin and counterstained with erythrosin. Sections from leaf (*a*) showed that one of the initial symptoms of infection is hypertrophy of the parenchyma cells adjoining the phloem (Fig. 10), and also necrosis of the sieve tubes and hypertrophy and hyperplasia of the adjacent parenchymatous tissues as shown in Figs. 11, 12 and 13. These effects were visible in many veins of the leaf. Fig. 14 represents a single vascular bundle from the midrib of leaf (*b*) showing phloem necrosis and disruption of the bundle cap at one side. Several sections from leaf (*b*) showed complete collapse of the parenchymatous cells adjoining the phloem tissue of the vascular strands (Fig. 15), which is obviously the condition underlying the severely " etched " appearance of the veins. The translucent effect would result from the hypertrophy and disintegration of chloroplasts in the mesophyll tissues seen in sections of leaf (*a*).

These effects resemble the primary effects of Curly Top of Sugar beet described by Esau (6). However, in the latter disease the necrosis is more severe and is usually succeeded by extensive proliferation and radial elongation of parenchyma cells external to the vascular bundles, resulting in the thickened veins and overgrowths which are a characteristic macroscopic symptom of the disease. There is also a conspicuous amount of phloem exudate. The work of Bennett and Esau (2) has shown that the Curly Top virus exists and multiplies probably exclusively in the phloem of affected host plants. In sugar beet affected by etch yellows, the above mentioned pathological changes, of which vein clearing is the external sign, are ephemeral. Their development, however, shows that a disturbance in the phloem is one of the earliest effects of the virus. Considering the nature of the subsequent symptoms of the disease, which Quanjer (10) and van Riemsdijk (12) have shown to be due to inhibited translocation, it seems probable that this phloem disturbance exists permanently in the affected plants, even though further visible changes in the phloem tissues, such as gummosis, may not take place until after the development of yellowing in the lower leaves.

ETCH YELLOWS IN WILD BEET (*Beta maritima*).

Roland (11) was the first to observe that virus yellows overwinters in the roots of *Beta maritima* and other workers have confirmed this observation. In 1947 Mr. B.

Crombie observed yellowing in wild beet growing in Co. Kerry in the extreme south-west of Éire. Specimens were forwarded to this laboratory for examination, but an immediate transmission test was impracticable as the plants withered completely after having been potted in the glasshouse. They were placed out-of-doors to recover and in due course made luxuriant growth, the lower leaves showing yellowing as before. Transmission tests from these plants to sugar beet and spinach, using *M. persicae* as vector, resulted in typical etch yellows symptoms in the host plants. Whether the etch yellows was present in the plants when originally received is not certain, as the infection might possibly have been contracted during the period mentioned above when the plants were out-of-doors. It is clear, however, that *Beta maritima* is susceptible to etch yellows.

Attempts to Infect Solanaceous Hosts with Virus Yellows.

Owing to the similarity in the physiological effects on the host plant of virus yellows and the leaf roll virus of potato, tests were made to see if potatoes could be infected with the sugar beet virus. For this purpose six sprouting tubers of the variety Arran Cairn and six of Dunbar Standard (both of which are very susceptible to leaf roll), were colonised with *Myzus persicae* from a sugar beet plant affected with etch yellows. A similar number of tubers was colonised with insects from a yellowed plant of family 41. After some days the insects were removed and the tubers grown on in the glasshouse. None of the resulting plants showed symptoms. Similar tests with *Nicotiana glutinosa, Nicotiana rustica* and *Datura stramonium* also gave negative results.

Acknowledgments.

The authors are greatly indebted to Professor R. McKay for his interest and helpfulness during the course of this investigation. They also wish to thank Mr. B. Crombie and other officials of the Irish Sugar Company who furnished them with specimens and information regarding the beet crops. The photographs were taken by Mr. G. H. McLean, A.R.P.S.

Summary.

Seed of a new family of sugar beet (No. 41), bred in Éire in 1945, when sown in an insect-proof glasshouse gave rise to plants a high percentage of which showed a yellows disease indistinguishable from virus yellows isolated from field crops of beet and mangold.

The disease was non-transmissible by sap inoculation to healthy sugar beet and spinach plants. Transmission was successfully accomplished in 6 different experiments using the aphis *Myzus persicae* (Sulz.) as vector. In 3 different experiments *Aphis Fabae* failed to transmit the virus.

The physiological effects of the disease in family 41 are similar to those of virus yellows. Starch accumulates in the toughened and yellow areas of affected leaves and is retained therein after the plants have been 48–72 hours in darkness.

Two types of yellows disease were isolated from commercial sugar beet crops in Éire. These are designated mild yellows and etch yellows, respectively.

In the case of mild yellows, the first symptoms to appear are a toughening and yellowing of the basal leaves. The yellowing spreads acropetally in the lower parts of the plant, the chlorotic areas subsequently becoming necrotic. The primary symptom of etch yellows consists of a clearing of the veins of the young developing leaves which become whitish or superficially necrotic. Subsequently the symptoms are similar to those of mild yellows, except that the chlorosis is more extensive and growth of the plant is more stunted. Minute reddish rings or spots which develop on the yellowed leaves are believed to be characteristic of the disease.

Both diseases are readily transmitted by *Myzus persicae*, but *Aphis Fabae* proved to be a poor and erratic vector. They are also transmitted by *Macrosiphum solanifolii*, but the comparative efficiency of this aphis as a vector of yellows has not been estimated.

No evidence was obtained that two different viruses were responsible for the vein clearing and yellowing symptoms in etch yellows. Although etch yellows can infect plants already containing mild yellows, the former is, nevertheless, regarded as a strain of the latter.

Examination of the internal anatomy of young sugar beet leaves showing vein clearing following infection with etch yellows revealed that the phloem tissues become necrotic and there is hypertrophy and hyperplasia of the surrounding parenchymatous cells. The latter may eventually collapse.

The wild beet (*Beta maritima*) is susceptible to etch yellows. Various solanaceous plants, including the potato (*Solanum tuberosum*) proved to be non-susceptible to etch and mild yellows.

References.

(1) BAWDEN, F. C. Plant viruses and virus diseases. Chronica Botanica Co., Waltham, Mass., U.S.A. 2nd Ed. 1943.
(2) BENNETT, C. W. and ESAU, K. J. Agr. Res. **53** : 595–620, 1936.
(3) CLINCH, P. E. M. Sci. Proc. Roy. Dublin Soc., **23** (N.S.) : 18–34, 1942.
(4) ———— *Ibid.*, **23** (N.S.) : 273–279, 1944.
(5) ————, LOUGHNANE, J. B. and MCKAY, R. Nature, Vol. 161, p. 28, Jan. 3, 1948.
(6) ESAU, K. Phytopath. **23** : 679–712, 1933.
(7) HALE, J. B., WATSON, M. A. and HULL, R. Anns. App. Biol. **33** : 13–28, 1946.
(8) HULL, R. and WATSON, M. A. Agric. J. Min. Agric. **52** : 66–70, 1945.
(9) KLINKENBERG, C. H. Meded. Inst. Suikerprod., Bergen-o-Z., **2** : 33–65, 1946.
(10) QUANJER, H. M. Tijdschr. PlZiekt., **40** : 201–214, 1934.
(11) ———— and ROLAND G. *Ibid*, **42** : 45–70, 1936.
(12) VAN RIEMSDIJK, J. F. *Ibid.*, **41** : 317–329, 1935.
(13) VAN SCHREVEN, D. A. Meded. Inst. Suikerbiet., Bergen-o-Z., **6** : 1–36, 1936.
(14) WATSON, M. A. Proc. Roy. Soc. Lond. B. **128** : 535–552, 1940.
(15) ———— — Ans. App. Biol., **29** : 358–365, 1942.

Explanation of Plates.

Plate 13.

Fig. 1. Lower leaf from sugar beet plant of family 41 showing general yellowing due to seed-borne virus.

Fig. 2. Leaf from affected plant of family 41 showing yellowing confined to tip. Note sharp demarcation of yellow and green areas.

Plate 14.

Fig. 3. Leaf from affected plant of family 41 showing asymmetrical development of yellowing.

Fig. 4. Lower leaf from plant of family 41 showing final stage of disease.

Plate 15.

Figs. 5 and 6. Lower leaves from affected plants of family 41 which had been kept in darkness for 48 hours. Decolorised and stained with iodine. Showing retention of starch in areas which had shown yellowing in fresh leaf.

Plate 16.

Fig. 7. Upper leaf from young sugar beet plant showing conspicuous vein clearing following infection with etch yellows.

Fig. 8. " Etching " of veins in young leaf of spinach (*Spinacia oleraceae*) following infection with etch yellows.

Fig. 9. Lower leaf of sugar beet plant affected with etch yellows showing numerous minute rings and spots. These were reddish in colour.

Plate 17.

Photomicrographs of sections of young leaves of sugar beet showing vein clearing following infection with etch yellows. Stained Heidenhain's iron alum haematoxylin and erythrosin.

Fig. 10. Hypertrophy of parenchymatous tissues adjoining phloem. This effect underlies the translucence or " clearing " of veins. X 258.

Figs. 11 and 12. Necrosis in phloem and hypertrophy and hyperplasia of adjoining parenchymatous cells. X 245 and 260, respectively.

Fig. 13. Transverse section through tip of leaf under low power showing necrosis in phloem and extending to surface. X 70.

Fig. 14. Vascular bundle from midrib of leaf showing necrosis of phloem at one side of bundle cap and hypertrophy of adjoining parenchymatous tissue. X 254.

Fig. 15. Section of leaf showing complete collapse of parenchymatous tissue adjoining the necrotic phloem. This effect gives rise to the severely " etched " appearance of the vein. X 178.

Fig. 2.

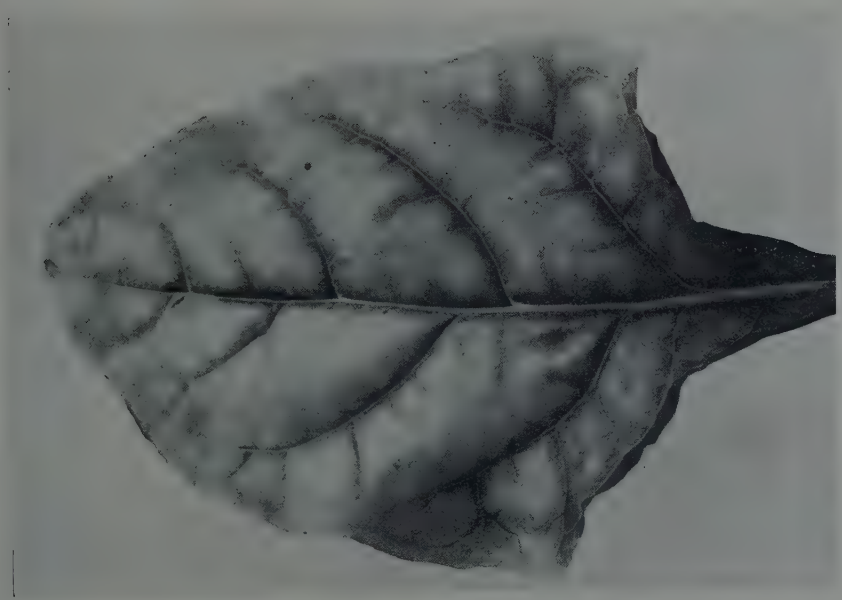


Fig. 1.

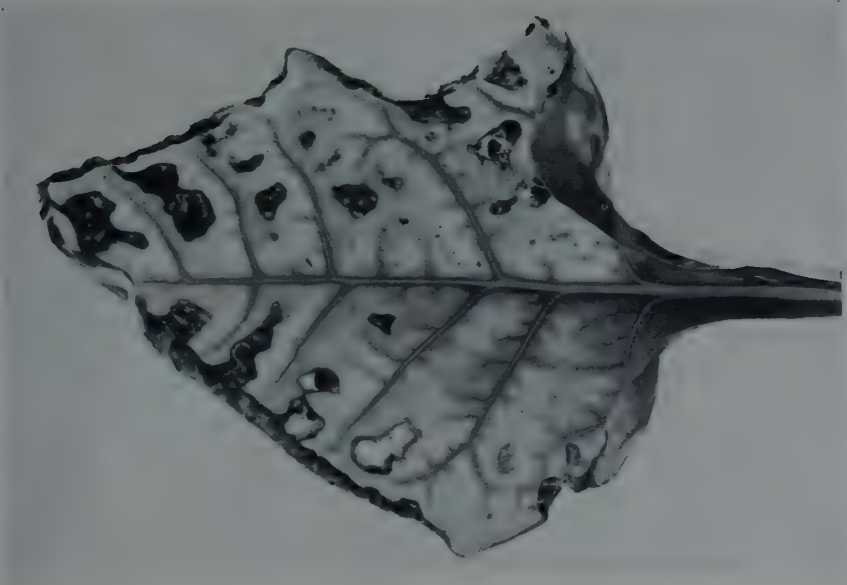


Fig. 4.

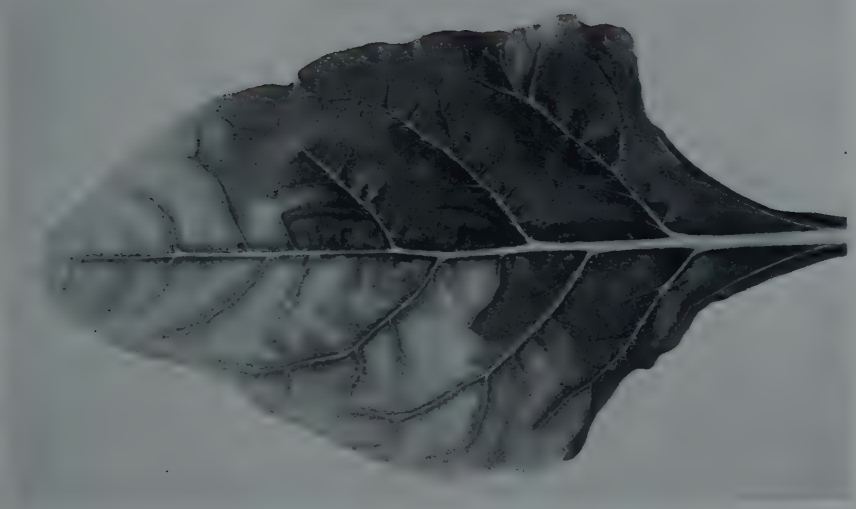


Fig. 3.

Fig. 6.

Fig. 5.

Fig. 9.

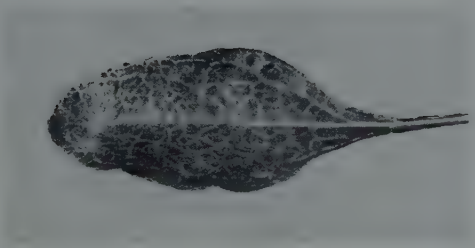


Fig. 8.

Fig. 7.

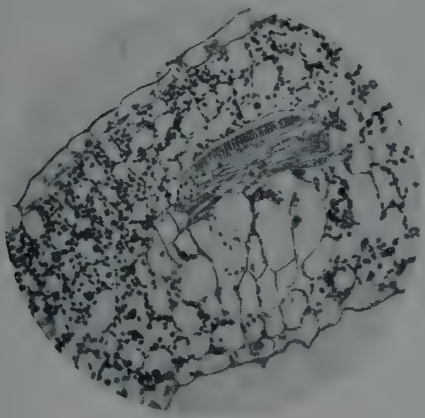


Fig. 10

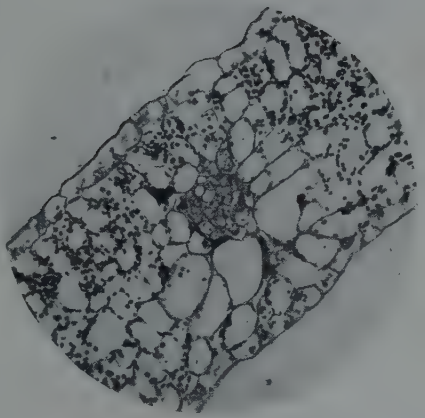


Fig. 11

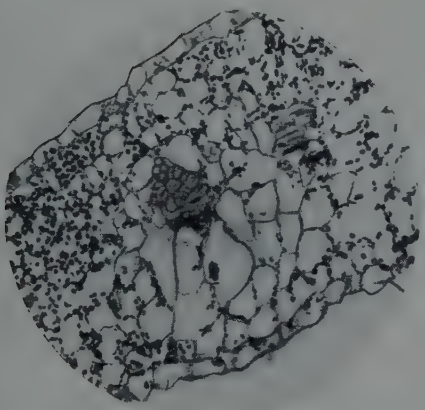


Fig. 12

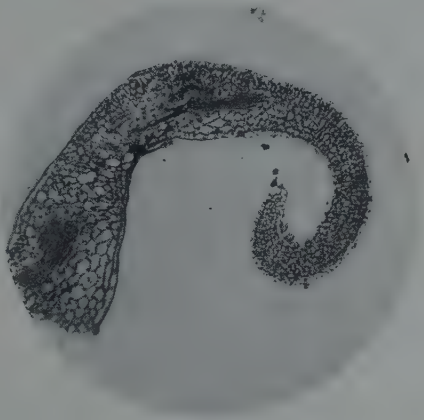


Fig. 13

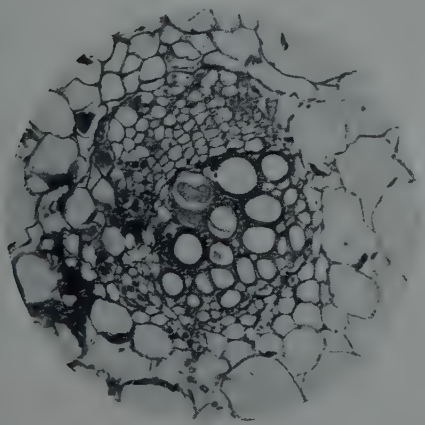


Fig. 14

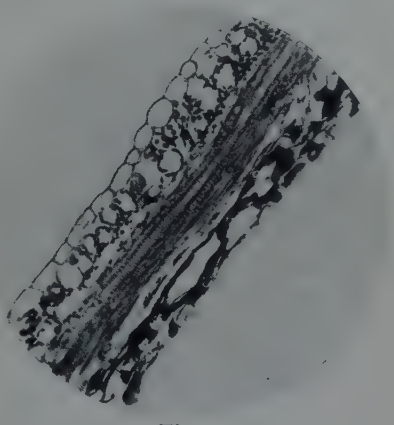


Fig. 15

No. 28.

THE CHEMICAL CONSTITUENTS OF LICHENS FOUND IN IRELAND.

BUELLIA CANESCENS. PART 3.

THE CONSTITUTION OF DIPLOICIN.

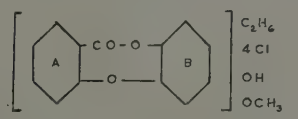
By

(The late) THOMAS J. NOLAN, JOSEPH ALGAR, EUGENE P. McCANN, WILLIAM A. MANAHAN AND NIALL NOLAN,

University College, Dublin.

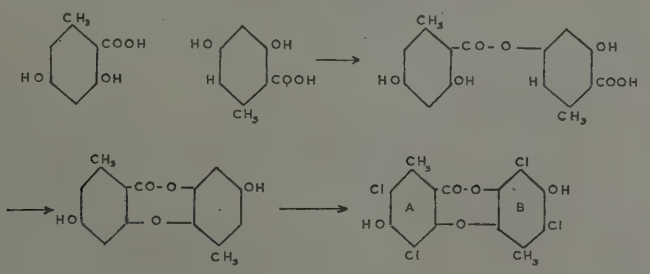
[Read MARCH 23. Published Seperately, SEPTEMBER 6, 1948.]

IN Part 2 of this investigation it was shown by Spillane, Keane, and Nolan (Sci. Proc. R.D.S. 1936, **21**, p. 333) that the lichen *Buellia Canescens* contains, amongst other substances, the compound diploicin, to which they gave the formula $C_{16}H_{10}O_5Cl_4$. It was also shown by these workers that diploicin is a depsidone and that to it could be attributed the structure :—



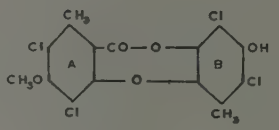
(I)

It was surmised that the C_2H_6 consisted of two methyl groups, rather than an ethyl group and a hydrogen atom, and that diploicin resulted from the condensation of two molecules of orsellinic acid, with subsequent elimination of one carboxyl group and simultaneous oxidation and chlorination. This would result in the compound II the monomethyl ether of which would be diploicin.



(II)

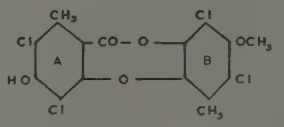
By analogy with known lichen substances it was regarded as most likely that the hydroxyl group in ring A had been methylated to form diploicin, which would accordingly have the structure III.



(III)

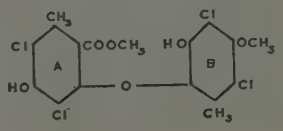
It should be noted that evidence so far given in proof of this configuration is limited to establishing the formula I, and that the complete formula III is based on analogy. A Kuhn-Roth determination of C-methyl has since been carried out and indicates the presence of two C-methyls, thus confirming the earlier surmise.

In the present communication, degradation experiments on diploicin are described which appear to prove that the structure of diploicin is correctly represented by the formula IV.



which places the methyl ether grouping in ring B and not in ring A, as was considered likely in the previously cited communication.

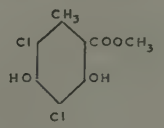
The orientation of the groups in ring A has been established in the following way. On treatment of diploicin with methyl alcoholic potash, the lactone bridge is opened to form the ester V (Spillane, Keane, and Nolan, *loc. cit.*).



(V)

2.4.3′.5′–*tetrachlor*—3.2′–*dihydroxy*—6–*carbomethoxy*—4′–*methoxy*—5.6′–*dimethyl diphenyl ether.*

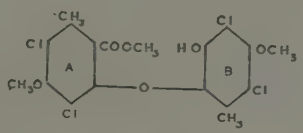
The breakdown of the ester V by fission of the oxygen bridge was accomplished in the following way. The ester was subjected to super-chlorination, followed by reduction and hydrolysis. This resulted in the production of 4..6–dichlor-orsellinic acid methyl ester VI.



(VI)

a substance which has been synthesised by Nolan and Murphy (Sci. Proc. R.D.S. 1940, **22,** p. 317). Control experiments in the case of gangaleoidin (Davidson, Keane, and Nolan, Sci. Proc. R.D.S. 1943, **23,** p. 143) had shown that super-chlorination does not result in demethylation, and consequently it was probable that the methoxyl group of diploicin, by inference, was in ring B. Definite proof of the occurrence of the free hydroxyl group in ring A was forthcoming from the following procedure.

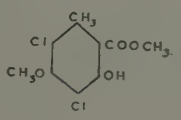
Diploicin was methylated, and the lactone bridge then opened to form the ester VII (Spillane, Keane, and Nolan *loc. cit.*).



(VII)

2.4.3′.5′–*tetrachlor*—2′–*hydroxy*—6–*carbomethoxy*—3.4′–*dimethoxy*—5.6′–*dimethyl diphenyl–ether.*

The ester VII was super-chlorinated, and on reduction and hydrolysis gave the mono-methyl ether of 4.6–dichlor–orsellinic acid methyl ester VIII.

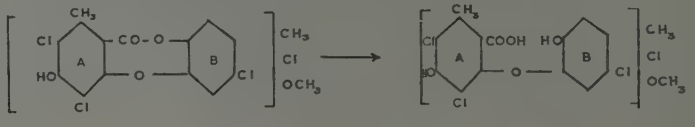


(VIII)

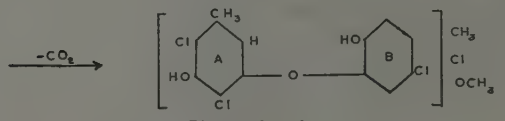
thus proving that the original methoxyl group in diploicin is in ring B and not in ring A. In the work so far described, no product was isolated which gave a clue to the orientation of the groups in ring B. It seemed likely that the most profitable method to isolate this half of the diploicin molecule, or a derivative thereof, would be to demethylate diploicin, and examine the nature of the product obtained, by submitting the resultant material to alkaline hydrolysis. Before doing so, the ester VII was examined for its colour reaction with 2.6–dichlorquinone–chlorimide in a sodium borate buffer solution. This reaction has been employed by Todd and Hems (J.C.S., 1940, p. 1208) as a test for a hydrogen atom para to a hydroxyl group in a benzene ring. Davidson, Keane, and Nolan (*loc. cit.*) have shown that it applies not only to hydrogen para to hydroxyl but also to certain negative substituents

para to hydroxyl. Thus, para chlorphenol gives an intense blue, para hydroxy benzoic acid a deep blue, but para hydroxy benzoic ester gives no definite colour reaction. Now diploicin gives no definite colour reaction, but when the lactone bridge is opened to form the ester VII, thus setting free a hydroxyl group in ring B, a deep blue colour is obtained on testing. This colour reaction is therefore strong presumptive evidence for the presence of a chlorine atom in ring B in a position para to the oxygen atom of the lactone bridge (see IX).

When diploicin is subjected to alkaline hydrolysis and the resulting product is subjected to further hydrolysis or heated above its melting point, a series of compounds is obtained which may be explained in the following way :—



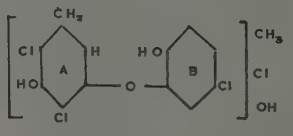
(IX)



(X)

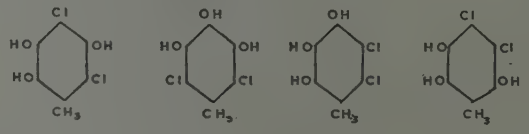
that is to say, a substituted di-tolyl ether is produced.

On the other hand, when diploicin is heated with hydrobromic acid in acetic acid solution, the lactone bridge is opened and the product is simultaneously decarboxylated and demethylated, giving rise to the substituted di-tolyl ether XI.



(XI)

The methylated di-tolyl ether X is resistant to the action of alcoholic potash, but the demethylated product XI is readily attacked, and fission of the oxygen bridge takes place. On the assumption that the process is hydrolytic, ring B in XI could give rise to the following products :—

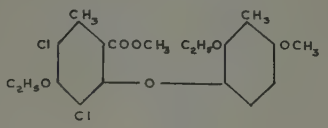


(XII) (XIII) (XIV) (XV)

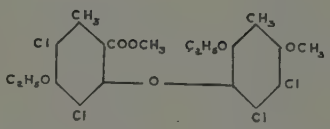
while ring A should give 4·6-dichlor-orcinol.

The structure XV, as a possible product, was excluded in the following manner by Nolan (see article by T. J. Nolan on Lichen Substances, Thorpe's Dictionary of Chemistry, 1946, p. 299).

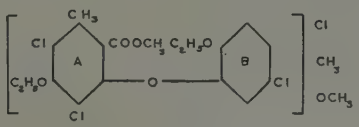
From gangaleoidin was prepared the substance



which on chlorination gave



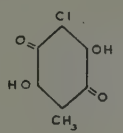
melting point 76°–77°C. which was not identical with the compound



melting point 94°–95°C., prepared from diploicin by treatment with methyl alcoholic potash, followed by treatment with diazo-ethane.

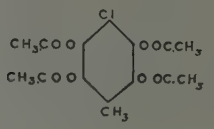
It follows therefore, that the possible products of hydrolysis arising from ring B are XII, XIII or XIV, which, in their turn, might by a process of oxidation give rise to a toluquinone containing chlorine and hydroxyl groups.

When the di-tolyl ether XI was subjected to the action of methyl alcoholic potash, in the presence of oxygen, the only pure product isolated was a mono-chlor di-hydroxy toluquinone $C_7H_5O_4Cl$. On the assumption that this toluquinone originated from 2·4–dichlor trihydroxy toluene XII it could have the formula



(XVI)

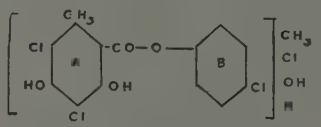
and, for reasons stated below, it seems certain that the substance is correctly represented by this structure. This toluquinone crystallises in very characteristic garnet-coloured hexagonal plates which give a violet coloration with a dilute solution of sodium carbonate. On reduction with sulphur dioxide and subsequent acetylation it gave the tetracetate of 4–chlor–2·3·5·6–tetrahydroxy toluene XVII, which melts at 219°C.



(XVII)

It was essential, therefore, to determine whether the toluquinone obtained from the di-tolyl ether XI, originated from 2·4–dichlor trihydroxy toluene XII, 2·6–dichlor trihydroxy toluene XIII, or 2·3–dichlor trihydroxy toluene XIV. The latter three compounds were prepared by us, and their synthesis is described in the experimental part. On examining their behaviour with methyl alcoholic potash, it was found impossible to isolate a toluquinone from the products of the reaction with 2·6–dichlor trihydroxy toluene or with 2·3–dichlor trihydroxy toluene. On the other hand, when 2·4–dichlor trihydroxy toluene was treated with methyl alcoholic potash, we were able to isolate a mono-chlor dihydroxy toluquinone, which crystallised in garnet coloured hexagonal plates and gave a violet coloration with dilute sodium carbonate. On reduction with sulphur dioxide and subsequent acetylation it gave the tetracetate of 4–chlor–2·3·5·6–tetrahydroxy toluene. M.P. 219°C.

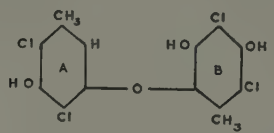
Further confirmation of the structure of diploicin was obtained in the following manner. When diploicin, in benzene solution, was heated with anhydrous aluminium chloride, a product was obtained which appeared to have the structure XVIII.



(XVIII)

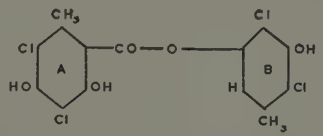
This substance formed a trimethyl ether and a triacetate, and on treatment with methyl alcoholic potash gave two products. One of these was the methyl ester of 4·6–dichlor orsellinic acid VI, and originated from ring A. The second product, derived from ring B, crystallised in garnet-coloured hexagons, identical with XVI, as on reduction and acetylation it gave the tetracetate of a mono-chlor tetrahydroxy toluene, M.P. 219°C., the mixed melting point of which with XVII was unchanged. These facts appear definitely to exclude the possibility of the toluquinone having its origin in ring A, since 4·6–dichlor orsellinic ester remains practically unaltered when treated with methyl alcoholic potash under similar conditions (see Nolan and Murphy, Sc. Proc. R.D.S. 1940, **33**, p. 317).

It is clear, therefore, that the same toluquinone was obtained by the action of methyl alcoholic potash on the di-tolyl ether XI, on the compound XVIII, and on 2·4–dichlor trihydroxy toluene XII. The groupings present in the latter substance must therefore be present in the di-tolyl ether XI, which may therefore be formulated



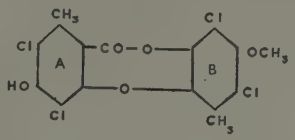
2·4·3′·5′–tetrachlor—3·2′·4′–trihydroxy—5·6′–dimethyl diphenyl ether

and the structure of XVIII may be represented by



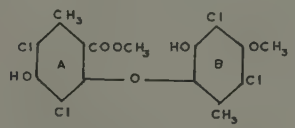
(*2·4–dichlor—3–hydroxy—5–methyl*) *phenyl ester of* 4·6*–dichlor–orsellinic acid.*

Consequently the formula for diploicin must be



EXPERIMENTAL PART.

Action of Chlorine followed by reduction and hydrolysis on the Compound V.



(M)

To a solution of the above compound (0·5 g.) in chloroform (60 c.c.) was added 13·3 c.c. of a solution of chlorine in carbon tetrachloride containing 0·04 g. chlorine per c.c. After standing for 24 hours in a stoppered flask, the excess chlorine was removed by blowing dry air through the solution, and the solvents removed by evaporation. The residue was a brownish gum. The latter was reduced in acetic acid solution (25 c.c.) with stannous chloride (1 g.) in twenty per cent. hydrochloric acid (30 c.c.). The latter solution was added in portions at a temperature of about 40°C. A precipitate was formed immediately. After standing overnight, water (50 c.c.) was added and the supernatant liquid decanted from the solid, which was then washed with a little five per cent. hydrochloric acid, followed by water, and air dried. The solid was refluxed gently with ten per cent. methyl alcoholic potash (25 c.c.) in an atmosphere of hydrogen for one hour. The solution was then diluted with water, acidified, and extracted with ether. The ether solution was successively extracted with three per cent. solutions of (*a*) sodium bicarbonate and (*b*) sodium carbonate.

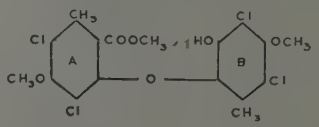
(*a*) The sodium bicarbonate extract was acidified and the precipitated material subjected to steam distillation, giving a white crystalline material in the distillate. On crystallisation of this material from aqueous methyl alcohol, colourless crystals were obtained M.P. 115°C., identical with the 4·6–dichlor orsellinic acid methyl ester (VI) prepared by Nolan and Murphy (*loc. cit.*).

Found :— C40·2, H3·8, Cl 26·0.

$C_9H_8O_4Cl_2$, H_2O requires :— C40·14, H3·71, Cl 26·4.

(*b*) Acidification of the sodium carbonate extract mentioned above, gave the original substance V, unaltered.

Action of Chlorine followed by reduction and hydrolysis on the Compound VII.



(VII)

The above compound (0·55 g.) was dissolved in chloroform (30 c.c.) and to it was added a solution of chlorine in carbon tetrachloride (6·5 c.c.) containing 0·085 g. of chlorine per c.c. The mixture was allowed to stand five days in a stoppered flask, excess chlorine was removed in a stream of dry air, and the solvents removed by evaporation. The residual oil was dissolved in acetic acid (40 c.c.) and reduced with stannous chloride (1·2 g.) dissolved in twenty per cent. hydrochloric acid (40 c.c.). After standing overnight, the mixture was diluted with water and extracted with chloroform. After washing with water, the chloroform solution was evaporated, leaving an oil. The latter was refluxed with ten per cent. methyl alcoholic potash (30 c.c.) for one hour, in a hydrogen atmosphere. The mixture thus obtained was cooled, diluted with water, acidified with dilute sulphuric acid, and extracted with ether. The ether solution was successively extracted with (*a*), three per cent. sodium bicarbonate and (*b*), one per cent. sodium hydroxide.

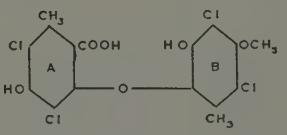
(*a*) Acidification of the sodium bicarbonate solution gave a brown gum which could not be obtained in a crystalline condition.

(*b*) The sodium hydroxide extract was acidified with dilute sulphuric acid and extracted with ether. Evaporation of the ether gave an oil which was repeatedly treated with hot petroleum ether (B.P. 60°–80°C.). On cooling, the solvent deposited crystals, which were recrystallised twice from aqueous methyl alcohol, giving colourless needles, M.P. 78°–79°C. The latter gave a violet coloration with ferric chloride, and the substance was identical with the synthetic 4·6–dichlor orsellinic acid methyl ester 5–monomethyl ether (VIII) described by Nolan and Murphy (*loc. cit.*).

Found :— C45·1, H3·9, OCH_3 23·9.

$C_8H_4O_2Cl_2(OCH_3)_2$ requires :—C45·3, H3·8, OCH_3 23·4.

2·4·3·'5'–tetrachlor—3·2'–dihydroxy—6–carboxy—4'–methoxy—5·6'–dimethyl diphenyl ether IX.



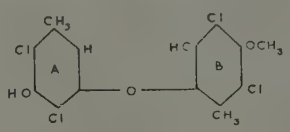
(IX)

Diploicin was hydrolysed by heating to gentle boiling with two per cent. aqueous sodium hydroxide. The liquor at first was pale yellow, afterwards slightly brownish. The heating was continued until practically all the solid had gone into solution (ca. fifteen minutes), then cooled, filtered, and acidified with dilute hydrochloric acid. The precipitate obtained was filtered, washed with water, and air dried. The product was crystallised several times from boiling benzene, and then dried in the air oven at 120°C. for several hours. The crystals thus obtained occurred both as colourless rhombohedra and as needles. M.P. 216°–218°C. (decomp.).

Found :— C43·93, H3·02.

$C_{16}H_{12}O_6Cl_4$ requires :— C43·44, H2·71.

2·4·3'·5'–tetrachlor—3·2'–dihydroxy—4'–methoxy—5·6'–dimethyl diphenyl ether X.



(X)

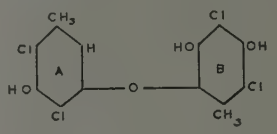
This compound was obtained by the elimination of carbon dioxide from the carboxylic acid IX above. Decarboxylation takes place very readily, and can be achieved in either of two ways.

Method I. The hydrolysis of diploicin described above was continued for a period of about one hour. When the solution thus obtained was saturated with carbon dioxide, most of the material was precipitated as a white solid, which did not contain a carboxyl group and had the di-tolyl ether structure X. Crystallisation from benzene gave colourless needles M.P. 186°C.

Method II. The carboxylic acid IX was heated above its melting point, when decomposition occurred with evolution of carbon dioxide. The resultant brown product was crystallised from dilute alcohol (norite) and afterwards from benzene, and was obtained as colourless needles M.P. 186°C., identical with the compound obtained by method I. The substance gives no colour with ferric chloride.

Found :— C45·45, H3·1, Cl35·4.
$C_{15}H_{12}O_4Cl_4$ requires :—C45·23, H3·02, Cl35·68.

2·4·3′·5′–tetrachlor—3·2′·4′–trihydroxy—5·6′–dimethyl diphenyl ether XI.



(XI)

This compound was produced by either of the following procedures.

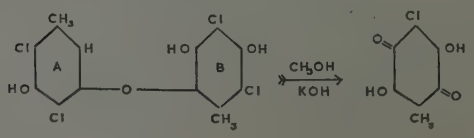
Method I. A mixture of the methoxy derivative X (9 g.), acetic acid (180 c.c.), constant boiling hydrobromic acid (150 c.c.) and red phosphorus (18 g.), was boiled under reflux for eight hours and was then cooled and filtered. The filtrate, on diluting largely with water, gave a small amount of cream precipitate. The residue of red phosphorus was extracted several times with boiling alcohol, filtered, and the alcoholic filtrate diluted with water, giving a considerable amount of white precipitate. The latter was crystallised from dilute alcohol, and furnished colourless, hexagonal crystals, M.P. 235°C.

Method II. Diploicin (1 g.) in acetic acid (20 c.c.) was heated with red phosphorus (2 g.) and constant boiling hydrobromic acid (16 c.c.) for eight hours, under reflux. After cooling the liquor was filtered, and the phosphorus washed with acetic acid. The whole of the filtrate was diluted with water and saturated with sodium bisulphite in order to decolorise it. A cream coloured precipitate was obtained. Further quantities of material were obtained by extraction of the phosphorus with hot alcohol. Crystallisation of the crude substance from hot dilute alcohol and boiling benzene gave colourless hexagons, M.P. 235°C. The mixed melting point of this substance with that obtained by method I was unchanged. Yield about 60 per cent.

Found :— C 44·53, H 2·96, Cl 36·35.
$C_{14}H_{10}O_4Cl_4$ requires :— C 43·7, H 2·6, Cl 37·0.

Action of methyl alcoholic potash on 2·4·3′·5′–tetrachlor—3·2′·4′–trihydroxy—5·6′–dimethyl diphenyl ether XI.

Production of the toluquinone XVI.



(XV) (XVI)

The compound XI (1·3 g.) was treated with five per cent. methyl alcoholic potash (30 c.c.), and heated under reflux at 60°C. for about three hours. Throughout this period, a slow stream of oxygen was bubbled through the solution. On standing overnight, the dark brown potassium salt of the quinone separated. This was filtered off, washed with a little methyl alcohol, dissolved in water, and acidified with dilute hydrochloric acid. The quinone was extracted with ether, the ether solution dried over anhydrous sodium sulphate, filtered, and evaporated to dryness, leaving a red crystalline residue. The latter was dissolved in the minimum volume of warm ethyl acetate, a large excess of chloroform added and the mixture allowed to stand. After a short time, garnet coloured hexagonal crystals of the quinone separated. The quinone sinters about 230°C., becomes black and melts about 264°C. (decomp.). The substance gives a violet colour with one per cent. aqueous sodium carbonate.

Found :— C 44·57, H 2·72, Cl 19..1.
$C_7H_5O_4Cl$ requires :— C 44.55, H 2.65, Cl 18.8.

Tetracetate of 4–chlor—2·3·5·6–tetrahydroxy toluene XVII.

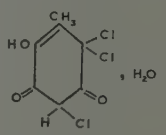
The toluquinone XVI was suspended in water, saturated with sulphur dioxide and the mixture allowed to stand for several days, the solution then having a very pale straw colour. Extraction of this solution with ether gave crude 4–chlor–2·3·5·6–tetrahydroxy toluene M.P. ca. 183°C., which tended to darken on exposure to the air. The air dried tetrahydroxy compound was dissolved in acetic anhydride to which a drop of concentrated sulphuric acid was added. After standing one hour, the mixture was diluted with water and extracted with ether. The ether solution was washed with water and dried over anhydrous sodium sulphate. The residue obtained on evaporation of the ether was crystallised from a mixture of benzene and ligroin and gave colourless prisms M.P. 219°C.

Found :— C 50·15, H 4·36, Cl 10·38.
$C_{15}H_{15}O_8Cl$ requires :— C 50·21, H 4·18, Cl 9·9.

2.4–dichlor—3·5·6–trihydroxy toluene XII.

To obtain this substance use was made of an observation of Heinrich, Meyer and Droschky (Ber. 37, 1904, p. 1427) that the action of hypochlorous acid on β–amino–orcin (which is 2–amino–orcin) gives rise to a product of formula $C_7H_7Cl_3O_4$. Heinrich did not attribute a structure to this substance, but the work of Zincke and his co-workers

(Zincke and Schurmann, Ann. 1918, 417, pp. 236–256) on the keto-chlorides suggested to us that it was a keto-chloride $C_7H_5Cl_3O_3$, H_2O to which the following formula, or one of similar type might be attributed :—



Heinrich's product was obtained by a modification of his method and on reduction with stannous chloride, it gave rise to a dichlor–trihydroxy toluene, for which the only possible formula appears to be XII above.

(*a*) *Chlorination of 2–amino–orcin.* 2–amino–orcin (4·7 g.) was added to a cooled mixture of acetic acid (18·8 c.c.) and concentrated hydrochloric acid (9·2 c.c.) and chlorine was passed through the mixture to saturation. The liquid was allowed to stand overnight and then poured into a dish and placed in a draught to remove excess chlorine. An equal volume of water was added and the solution cooled, when the yellow crystalline compound which was precipitated was filtered, washed with a little water and dried over sodium hydroxide in vacuo. (yield 2·2 g.). It was crystallised from benzene giving colourless crystals (0·6 g.) M.P. 92°–95°C. On addition of a little water to the benzene mother liquors, colourless needles of a hydrate crystallised out (0·65 g.). Softens 92°C., M.P. 96°–98°C.

Analysis of this hydrate gave the following results :—

Found :— C 31·0, H 2·75, Cl 38·75.

$C_7H_5Cl_3O_3$, $1\frac{1}{2}$ H_2O requires :— C 31·05, H 2·88, Cl 39·4.

(*b*) *Reduction of the keto-chloride.* The keto-chloride (0.8 g.) was dissolved in acetic acid (4 c.c.). To the solution stannous chloride (2·8 g.) in concentrated hydrochloric acid (10 c.c.) was added slowly, with cooling. A white crystalline mass separated. The mixture was allowed to stand overnight, in the dark, in a stoppered flask when the crystals were filtered, washed with 1 : 1 hydrochloric acid-water and air dried. Recrystallisation from benzene gave pale pinkish-brown needles M.P. 168°–172°C. The substance gives a port-wine colour with ferric chloride and a cerise coloration with one per cent. sodium carbonate.

Found :— C 40·3, H 2·92, Cl 33·5.

$C_7H_6O_3Cl_2$ requires :— C 40·2, H 2·87, Cl 34·0.

Action of methyl alcoholic potash on 2·4–dichlor–3·5·6–trihydroxy toluene XII.

Production of the toluquinone XVI.

2·4–dichlor–3·5·6–trihydroxy toluene (3 g.) was dissolved in five per cent. methyl alcoholic potash (60 c.c.). A transient green colour was produced which

rapidly changed to deep purple. The solution was heated under reflux at 40°–50°C. for two hours, while a slow stream of oxygen was bubbled through. On standing overnight, the dark brown precipitate which had separated was filtered, washed with a little methyl alcohol, dissolved in water and acidified with dilute hydrochloric acid. The liquor was extracted with ether, the ether dried over anhydrous sodium sulphate and evaporated, leaving a reddish residue. The residue on crystallisation from ethyl acetate-chloroform gave garnet coloured hexagonal crystals sintering about 230°C., which gave a violet coloration with one per cent. sodium carbonate.

Found :— C 44·24, H 3·2, Cl 18·7.

$C_7H_5O_4Cl$ requires :— C 44·55, H 2·65, Cl 18·8.

The identity of this substance with the toluquinone XVI, obtained by the action of methyl alcoholic potash on the di-tolyl ether XI, was shown in the following way. Reduction of the substance followed by acetylation gave the tetracetate of 4–chlor–2·3·5·6–tetrahydroxy toluene M.P. 219°C.

Found :— Cl 9·36, $C_{15}H_{15}O_8Cl$ requires :— Cl 9·9.

The mixed melting point of this tetracetate with XVII was unaltered.

2·6–*dichlor*—3·4·5–*trihydroxy toluene XIII.*

In the production of this compound, considerable difficulty was experienced in preparing the necessary starting substance, 3·4·5–trihydroxy toluene. Various methods were tried of which the following two proved the most satisfactory.

Method I. 3·4·5–trihydroxy toluene was prepared from atranol (2·6–dihydroxy–4–methyl benzaldehyde) by the Dakin reaction in accordance with the procedure described by St. Pfau (Helv. Chim. Acta, 9, p. 667) and was obtained as brown needles M.P. 125°–130°C. Yield 25 per cent.

Method II. The procedure described by Asahina (Ber. 69, 1936, p. 2328) was more convenient and gave somewhat better yields than method I. Galloyl chloride trimethyl ether was reduced by Rosenmund's method to gallic aldehyde trimethyl ether. The latter was reduced by hydrazine hydrate to 3·4·5–trimethoxy toluene which was then demethylated with acetic acid–hydrobromic acid and furnished brown needles of 3·4·5–trihydroxy toluene M.P. 119°C.

Chlorination of 3·4·5–*trihydroxy toluene.* 3·4·5–trihydroxy toluene (1 g.) prepared by either of the above methods, was dissolved in dry ether (15 c.c.) and sulphuryl chloride (2·1 g. in 5 c.c. ether) was added. A maroon colour developed and slight evolution of heat took place. After standing one hour, the solution was shaken with a little water. The ether solution was dried over anhydrous sodium sulphate, filtered, and evaporated in vacuo, leaving a pale brown residue. Crystallisation from benzene gave 0·6 g. pinkish white crystals, which did not melt sharply, but over a range of 170°–180°C.

Found :— Cl 33·7, $C_7H_6O_3Cl_2$ requires :— Cl 33·9.

With one per cent. sodium carbonate 2·6–dichlor–3·4·5–trihydroxy toluene gave a cerise coloration, turning brown on standing. With aqueous ferric chloride it gave a cobalt blue colour, fading to pale blue on standing.

Action of methyl alcoholic potash on 2·6–dichlor–3·4·5–trihydroxy toluene XIII.

2·6–dichlor–3·4..5–trihydroxy toluene was treated with five per cent. methyl alcoholic potash. The solution, initially pink, changed to blue and finally brown. The mixture was heated under the same conditions as described for the treatment of 2·4–dichlor–3·5·6–trihydroxy toluene above and the separated solid worked up in a similar manner. The only product obtained was a brown oil, which could not be induced to crystallise. No material of quinone type could be isolated from the reaction mixture.

2·3–dichlor–4·5·6–trihydroxy toluene XIV.

2·3·4–trihydroxy toluene was obtained by Clemmensen reduction of pyrogallol o-aldehyde (Dimroth and Zoeppritz, Ber. 35, 1902, p. 996) according to the method of Majima and Okazaki (Ber. 49, 1916, p. 1492) and crystallised from benzene in off white needles M.P. 140°C. Chlorination of this trihydroxy toluene was effected with sulphuryl chloride as described for the production of XIII. Chlorination did not appear to proceed so smoothly as in the latter case, but material was obtained which crystallised from benzene in faintly pink oval plates, M.P. 120°C. (decomp.). With one per cent. sodium carbonate it gave a yellow colour, turning brown rapidly. Alcoholic ferric chloride gave a pale green coloration, turning olive green rapidly.

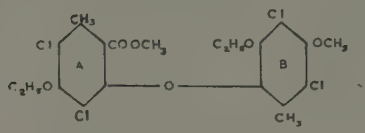
Found :— Cl 36·7, $C_7H_6O_3Cl_2$ requires :—Cl 33·9.

Although somewhat impure, the substance appeared to be the required dichloro derivative and was employed, without further purification in the following experiment.

Action of methyl alcoholic potash o 2·3–dichlor–4·5·6–trihydroxy toluene XIV.

2·3–dichlor–4·5·6–trihydroxy toluene was warmed with five per cent. methyl alcoholic potash under conditions similar to those described above for XII and XIII. Only brown oily material, which could not be induced to crystallise, was isolated from the reaction product. There was no indication of the formation of a quinone.

2·4·3′·5′–tetrachlor—3·2′–diethoxy—4′–methoxy—6–carbomethoxy—5·6′–dimethyl diphenyl ether.



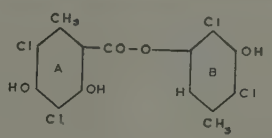
Treatment of the substance V with diazo-ethane and crystallisation of the product from methyl alcohol gave the above compound as colourless crystals M.P. 94°–95°C.

Found :— C 48·63, H 4·1, Cl 27·9.

$C_{15}H_6O_2Cl_4(OCH_3)_2(OC_2H_5)_2$ requires :— C 49·2, H 4·3, Cl 27·7.

This compound cannot be identical with the tetrachloro derivative obtained from gangaleoidin and mentioned in the introductory part, as the latter melts at 76°–77°C.

(*2·4–dichlor—3–hydroxy—5–methyl*) *phenylester of 4..6–dichlor—orsellinic acid XVIII.*



Diploicin (5 grms.) was dissolved in the minimum volume of boiling dry benzene anhydrous aluminium chloride (7 grms.) was added and the mixture refluxed on the steam bath for six hours. After standing overnight, the solvent was decanted and the residue decomposed with ice and dilute hydrochloric acid. It was then extracted with ether and the ether solution extracted several times with dilute sodium bicarbonate. Acidification of the sodium bicarbonate solution gave a white precipitate, which was washed with water, air dried and crystallised from benzene, in which it was sparingly soluble. The pure substance crystallises in pale yellow prisms M.P. 206°C. Yield 2·8 grms. The compound gives a faint reddish brown colour with ferric chloride and a yellow colour with bleaching powder solution. It dissolves in dilute sodium carbonate to a faintly yellow solution which does not give a violet colour on standing.

Found :— C 42·09, H 2·52, Cl 32·6.

$C_{15}H_{10}O_6Cl_4$ requires :— C 42·05, H 2·33, Cl 33·1.

Diploicin was recovered unchanged from the material insoluble in dilute sodium bicarbonate.

Acetylation of the compound with acetic anhydride and a trace of sulphuric acid gave a triacetate which crystallised from benzene and ligroin in colourless prisms M.P. 175°–176°C.

Found :— C 45·99, H 2·89, Cl 25·8.

$C_{15}H_7O_6Cl_4(CH_3CO)_3$ requires :— C 45·49, H 2·88, Cl 25·7.

Methylation of the compound with diazo-methane gave a trimethyl ether which crystallised from methyl alcohol in colourless prisms M.P. 184°C.

Found :— C 47·36, H 2·92, Cl 31·3.

$C_{15}H_7O_2Cl_4(OCH_3)_3$ requires :— C 47·57, H 3·5, Cl 31·2.

Action of methyl alcoholic potash on the compound XVIII.

Compound XVIII (1·3 grms.) in five per cent. methyl alcoholic potash (40 c.c.) was heated at 60°–70°C. for four hours while a slow stream of oxygen was bubbled through the solution. After standing overnight, the mother liquor was filtered from precipitated dark brown solid. The latter was dissolved in a little water, acidified with dilute hydrochloric acid and extracted with ether. Evaporation of the ether gave a reddish brown residue which crystallised from ethyl acetate-chloroform in garnet coloured hexagons (0·1 grm.), which sintered at 230°C. The identity of this compound with the toluquinone obtained from the ditolyl ether XI and from 2·4-dichlor–trihydroxy toluene XII was shown in the following manner. The substance was reduced with aqueous sulphur dioxide and the resultant product acetylated. The tetracetate thus obtained melted at 219°C. and was identical (mixed melt) with the compound XVII previously described.

The mother liquor, above, from the dark brown solid was saturated with carbon dioxide, diluted with water and extracted with ether. On evaporation of the ether and crystallisation of the residue from aqueous alcohol, colourless needles were obtained M.P. 120°–121C°., giving a violet coloration with ferric chloride. Analysis showed this substance to be the methyl ester of 4·6–dichlor–orsellinic acid VI, containing one molecule of water of crystallisation. A mixed melting point with the methyl ester of 4·6–dichlor–orsellinic acid (M.P. 115°–117°C.) synthesised by Nolan and Murphy (*loc. cit.*) melted at 115°–117°C.

Found :— C 39·48, H 4·01, Cl 26·3.

$C_9H_8O_4Cl_2$, H_2O requires :— C 40·14, H 3·71, Cl 26·4.

That this substance was actually the methyl ester of 4·6–dichlor–orsellinic acid VI was shown in the following way. The compound was acetylated and gave a diacetate which crystallised from aqueous alcohol in colourless prisms M.P. 93°C. and gave no colour with ferric chloride.

Found :— C 46·66, H 3·44, Cl 21·6.

$C_9H_6O_4Cl_2(CH_3CO)_2$ requires :— C 46·56, H 3·58, Cl 21·2.

The mixed melting point of this diacetate with the diacetate of 4·6–dichlor–orsellinic acid methyl ester, prepared in the manner described below, was unaltered.

A specimen of o-orsellinic acid methyl ester from lecanoric acid was chlorinated with sulphuryl chloride to give crude 4·6–dichlor–orsellinic acid methyl ester (M.P. ca 115°C.). The latter was acetylated and gave on crystallisation from aqueous alcohol a diacetate M.P. 93°C.

No. 29.

SALMON AND SEA TROUT OF THE RIVER INNY.

By

ARTHUR E. J. WENT.

(Plate 18.)

(Read November 25, 1947. Published Seperately, September 6, 1948).

The River Inny in County Kerry is a small spate river, about 16 miles long, with a catchment area of only 47 square miles (see Fig. 1). Its catchment area is entirely on the Old Red Sandstone, and it drains some very high ground (over 2,000 feet). As limestone is absent from the catchment area the waters of the river are always acid, the extent of the acidity depending to some degree on the height of the water. An analysis of the waters of the river when it was at an average level is given in Table 1. (See Appendix for all tables.)

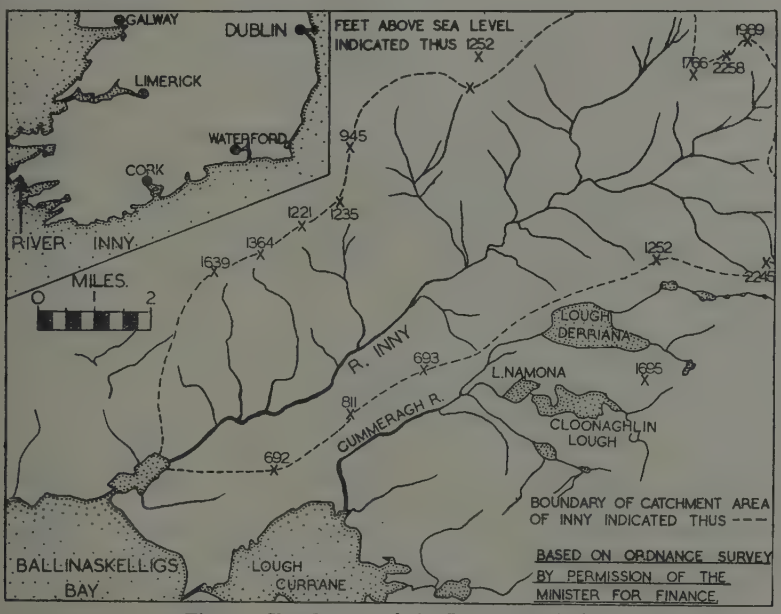


Fig. 1. Sketch map of the Inny watershed.

Apart from affording sport to the angler the Inny supported a commercial net fishery, partly in fresh water and partly in the tideway. Netting in the freshwater portion of the river has, however, been abolished under the provisions of Section 35 of the Fisheries Act, 1939, as from 1st January, 1948. The material on which this investigation was based was collected in the years 1941, 1942, 1943 and 1944 by the owner of one of the nets fishing in the river, and consisted of 747 satisfactory sets of scales and data relating to salmon, and 217 sets relating to sea trout. Unfortunately it was not found possible to weigh the fish. The distribution of the samples over the various months of the season is indicated in Table 2.

Age and Growth of the Salmon.

Smolt ages. The distribution of the various smolt classes has been given in Table 3. Four smolt classes were present. The proportion of the three-year smolt class was very high when compared with the results from other Irish rivers (see Went, 1947, *Sci. Proc. R. Dublin Soc.* 24 (N.S.) No. 19 p. 171). The proportion of old smolts (three and four years old) tends to decrease as the age groups are ascended. In other words there was a decrease in the average age of the smolt as the age groups were ascended.

Smolt types. When dealing with salmon of other Irish rivers (see bibliography, Went, 1947. *op. cit.*) it was mentioned that the smolts could be divided into two types, namely, (1) Type B. smolts which showed two or more growth elements on the scales corresponding to growth in fresh water in the spring prior to migration to the sea as a smolt, and (2) Type A. smolts which made no such growth. In the present material a similar division was made and the results are given in Table 4.

Age groups. The fish were divided into three groups of maiden or unspawned fish and one group of fish which had only one common character, namely the presence of one or more spawning marks on their scales. The results have been given in Table 5. The bulk of the fish were grilse (1 + winters) with the other three age groups each forming 7.0% or less of the total. The proportion of previous spawners was low (3.1%), being considerably lower than the average for Ireland in 1944 and 1945 (see Went, 1947, *op. cit.* p. 175).

May was the best month for small spring and summer fish (2 and 2 + winters) and July for grilse and previous spawners (Table 6). In April all the fish were spring fish, in May 39.1% were spring fish, in June 10.8%, and thereafter the runs consisted almost entirely of summer fish (Table 7).

Twenty-three previous spawners were examined, and they all exhibited the "short absence" habit, that is to say, the period feeding in the sea between successive entries into fresh water was less than a full year. Twenty-one of the previous spawners had spawned once previously, one twice, and one three times. The fish which had spawned three times previously, measured 35 inches in length, and was captured on 19 July, 1941. Fish of this type in Ireland are exceedingly rare. As

mentioned in a previous publication (Went, " The Value of the kelt " *Salmon and Trout Magazine* No. 119, Jan. 1947. p. 45) only two previous specimens have been recorded from Ireland. A photograph of one of the scales of this fish is given in Plate 18.

Divided migration and return. In Table 8 the years in which the different fish were hatched have been indicated. Five-year classes, i.e. the broods of five years, were responsible for the runs in one year, but over 96% of the fish belonged to two year-classes.

Size distribution. The distribution of the various size groups has been given in Table 9, and is illustrated in Fig. 2. As would have been expected in a grilse river the fish were concentrated in a few length groups, almost 3/4 of the fish having lengths between 21.95 and 26.95 inches.

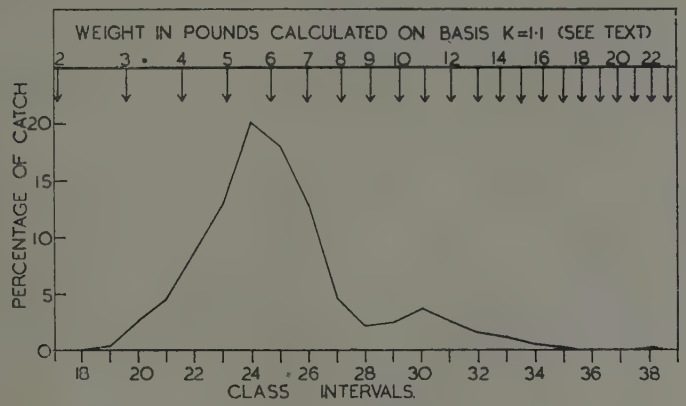

WEIGHT IN POUNDS CALCULATED ON BASIS K=1·1 (SEE TEXT)
2 3 4 5 6 7 8 9 10 12 14 16 18 20 22
PERCENTAGE OF CATCH
20
15
10
5
0
18 20 22 24 26 28 30 32 34 36 38
CLASS INTERVALS.

Fig. 2. Estimated size distribution of the Inny salmon.

Average sizes. Unfortunately the person collecting the material was not prepared to weigh the fish, and therefore we have had to be content with the length measurements only. Details of the average lengths in the different smolt classes of the different age groups are given in Tables 10–12, no attempt having been made to give the monthly averages, except in the case of the grilse, as the numbers of other age groups were small.

(a) Grilse (1 + winters). Number examined = 640.

	Inches	Date of Capture	Smolt Class
Minimum	18.6	3/7/'42	2 year
Average	24.5	——	——
Maximum	30.3	19/7/'41	2 year
	30.3	15/9/'41	2 year

From June onwards the average length of the grilse increased slightly as the season progressed.

(b) Small spring fish (2 winters). Number examined = 32.

	Inches	Date of Capture	Smolt Class
Minimum	26.4	11/5/'43	2 year
Average	32.4	——	——
Maximum	35.1	30/5/'41	2 year

(c) Small summer fish (2 + winters). Number examined = 52.

	Inches	Date of Capture	Smolt Class
Minimum	27.5	7/6/'41	2 year
Average	31.4	——	——
Maximum	38.0	15/9/'41	2 year

The largest fish of this age group was the largest fish of which scales were examined in this investigation and probably weighed about 22 pounds. (See Fig. 2.)

(d) Previous spawners (with SMs). Number examined = 23.

	Inches	Date of Capture	Smolt Class
Minimum	24.3	23/6/'44	1 year
Average	29.4	——	——
Maximum	35.0	19/7/'41	2 year

The smallest previous spawner had spawned only once previously, whereas the largest fish of this type had spawned three times previously (see page 336 and Plate 18).

In order to give some idea of the weights of the fish involved a rough scale has been added to Fig. 2. This scale has been drawn up on the basis of a value of 1.1 for the *Condition Coefficient* on Menzie's scale, the *Condition Coefficient* (K) being calculated from the formula $K = 100{,}000W/L^3 \times 36$. (W = weight in lbs. and L = length in inches).

Erosion. After a sojourn in fresh water the material of a scale of a salmon is partly absorbed, particularly at its margin. In the present material a proportion of the fish had eroded scales (see Table 13). In each age group the proportion of fish having eroded scales and the average degree of erosion increased as the season progressed. Erosion in the grilse (1 + winters) was not as advanced as in the other age groups.

Calculated lengths. The lengths of each maiden or unspawned fish at the end of every year of life were calculated in the usual way from the measurements of the scales, i.e. on the assumption that the growth of the scale is strictly in proportion to the growth of the fish.

River life. The relationships of the parr lengths are dealt with in this section. In Table 14 the growth in freshwater has been indicated for each smolt class (see also Fig. 3.). The fastest growing parr migrated first. At the end of the first year the one-year smolts were markedly longer than those of the other three smolt classes. Similar conditions have already been described for other Irish rivers (see bibliography Went, 1947, *op. cit.*). The length of the smolt was also calculated, and it

was shown that the mean smolt length increased as the smolt classes were ascended. (Table 14). The distribution curves for the two year smolt class have been added to Fig. 3.

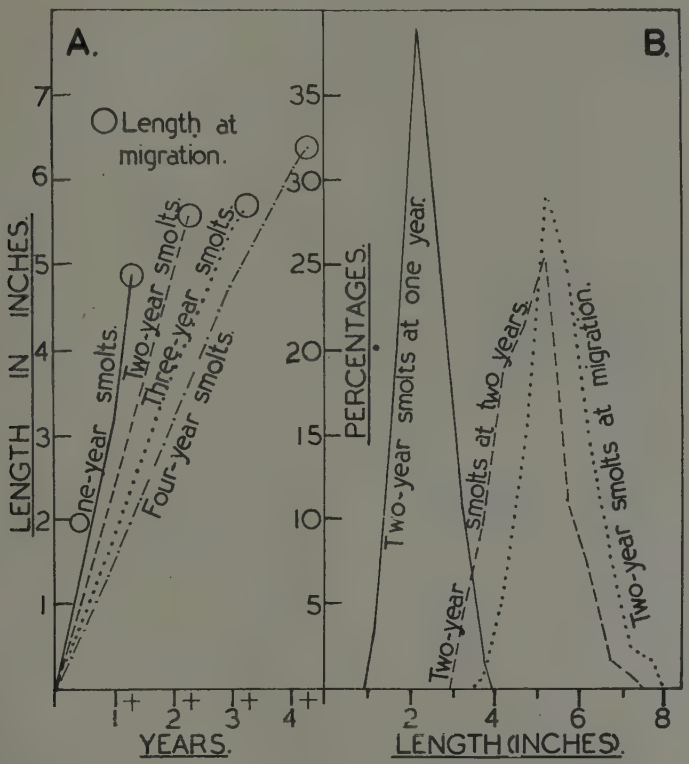


Fig. 3. A. Growth of Inny salmon in fresh water.
B. Distribution curves for the two-year smolts at one-year, at two years and at migrations as percentages of the total.

As was mentioned on page 336 the smolts could be divided into two types namely Type A and Type B smolts. The growth in each type in each smolt class is given in Table 15. In general the Type A smolts were longer at the end of any one year than the Type B smolts of the same year class.

In Table 12 the growth in fresh water of the different age groups has been given, and there appears to be no marked differentiation between the different age groups.

Sea life. The relationships of the lengths at the end of every year of sea life are dealt with in this section. In Tables 11 and 12 the lengths of the different age groups at the end of every year of sea life have been given. Taken as a whole the grilse had slightly superior lengths at the end of the first sea winter, but the differences are small and may not be significant. At the end of the second sea winter

the small spring fish (2 winters) were longer than the small summer fish, (2 + winters), so much so in fact that although on the average, the small summer fish put on 2.4 inches between the end of the winter and capture, they were still smaller than the small spring fish.

Notes on the Sea Trout.

As mentioned earlier a small amount of material relating to sea trout was collected from the River Inny during the years 1941 to 1944. This material, consisting of sets of scales with relevant data from 217 fish, cannot be regarded as completely adequate, quite apart from the question of its paucity. The fish, from which the material was obtained were all captured by means of a draft net with a mesh of 7 inches in the round ($1\frac{3}{4}$ inches knot to knot). Such a net is clearly selective, as many of the smaller sea trout, particularly whitling or finnock (fish in their first post-migration year) escape capture. Nevertheless bearing this in mind the results appear to be worth recording.

Smolt ages. In Table 16 the distribution of the different smolt ages in the various age groups has been given for the maiden or unspawned fish and the previous spawners together. The two-year smolts were the most important forming over three-quarters of the total, the second most important class being the three-year smolts (about one-seventh of the total). The proportion of one-year smolts was very high (cf. G.H. Nall, 1930. *The life of the sea trout* p. 311) but it must be remembered that this river is much further south than the majority of rivers investigated heretofore.

Smolt types. When dealing with the salmon (page 336) it was mentioned that the fish could be divided into two types on the basis of the growth made in fresh water in the spring before migration to the sea as a smolt. A similar division can be made in the sea trout also (Table 17). The bulk of the fish belonged to the type B smolts and the proportion of type A smolts increased as the smolt classes were ascended (Table 17).

Age groups. In Table 18 the fish have been classified on the basis of the period of sea feeding. It will be noted that fish in their second post migration year were in the majority. The relative absence of whitling or finnock (fish in their first post-migration year) to some extent, may have been due, as explained previously, to the selective capture of the fish. This is confirmed by consideration of the previous spawners from which it was estimated that about 20% had originally spawned in their first post-migration year (Table 18). Fish in their third or fourth post-migration year were clearly unimportant.

The monthly distribution into the various age groups of the fish investigated has been given in Table 19. In general the proportion of previous spawners tends to decrease as the season progresses. Although there was, in the material at the writer's disposal, an increase in the proportion of previous spawners in July, the

large incoming populations of small maiden fish, not sampled, tend to provide runs of fish almost completely maiden in character, as we know from our observations on this river.

In the material at the writer's disposal 70.5% of the fish were maiden fish, and the remainder were previous spawners. Of the previous spawners, 64 in number, 38 (59%) had one spawning mark, 20 (31%) had two such marks and 6 (10%) had three marks. There is a suggestion (Table 16) that the older smolts tend to return to the fresh water earlier than the younger smolt groups.

Dates of the incoming populations. In Ireland it is generally assumed that there are no worth-while runs of sea-trout in most of our rivers until the end of June. Whilst this may be so in some rivers, it is obvious that in the Inny there are clean-sea-trout entering the river from the beginning of April onwards. In fact the peak of the runs of large fish would appear to be in May (Table 19).

Divided migration and return. The years in which the fish were hatched have been indicated in Table 20. In the material at the writer's disposal the fish were derived from five spawning years. Owing to the absence of the younger smolt classes in the whitling (fish in their first post-migration year) the progeny of one other spawning may have been eliminated.

Average sizes. The average sizes in the various categories of fish have been given in Table 21. The symbols used in the first column of this Table require some explanation. The first figure indicates the period of feeding in fresh water before the smolt migration, the figure or symbol after the full stop indicates the period of sea feeding (+ = fish in their first post-migration year, 1 + in their second post-migration year, etc.) and the symbols SM+ which follow indicate the number of times a fish has spawned and returned to the fresh water after a period of sea feeding. After spawning the growth rate, as might have been expected, tended to fall off very much. This is partly due to the necessity of recovering material lost at spawning, and partly due to the fact that between spawnings the period of feeding in any one year is limited to two or three months. For example, as will be seen later, between the end of the first sea winter and the date of capture, the 2.1 + category increases in length from 12.6 inches to 16.4 inches (3.8 inches) whereas between the first spawning and the second return to fresh water as a clean sea trout the increment was only 1.2 inches (from 16.4 to 17.6 inches).

In the whitling (fish in their first post-migration year) the smolt age would appear to have a considerable influence on the size of the fish. For that reason only the whitling derived from old smolts appear to be taken by the draft net used in the River Inny. In those fish which have spent longer feeding in the sea before returning to fresh water to spawn, the smolt age has a much smaller effect on the mean length of the fish (cf. 2.1+ and 3.1+ fish in which the mean lengths are 16.4 and 16.5 inches respectively. Taking all the smolt classes together the mean lengths are as follows.

Age group	*No.*	*Mean length in inches*
+	3	13.4
1+	142	16.3
2+	6	18.1
3+	1	17.5

Oldest and longest fish. Nine fish in their seventh year were the oldest fish examined in this material. One of these, a fish whose life history may be indicated 3.1 + .2SM +, had a length of 23.5 inches, which was the highest length recorded from the sea trout examined. On the basis of a condition coefficient of 1 on the Scottish standard (cf. Nall, 1930, *op. cit.* p. 315) the fish would have weighed just under 6 lb. (5.97 lb.).

Calculated lengths. The length at the end of every year of life was calculated in the normal way, i.e. on the assumption that the growth of the scale was strictly in proportion to the growth of the fish, for every maiden fish of which the scales were suitable for measurements.

River Life. The mean length in inches in the different smolt classes at the end of each year of freshwater life have been given in Table 23. As in the case of the salmon the fastest growing parr migrated first. (see also Fig. 4). The mean smolt length increased as the smolt classes were ascended (Fig. 4). The mean smolt length at 7.5 inches is considerably lower than that of the Waterville River which has been estimated to be 9.9 inches (see Went, 1944 *Sci. Proc. R. Dublin Soc.* 23 (N.S.) No. 20 page 202). Type A smolts in general grow somewhat faster than type B smolts of the same smolt class.

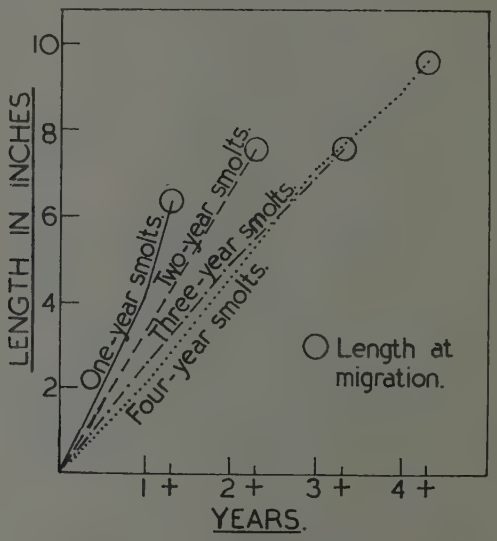


Fig. 4. Growth in fresh water of Inny sea trout.

Sea Life. Mean lengths at the end of each year in the sea in the different age groups have been given in Tables 23 and 24. As only the fish in their second post-migration year were in sufficient numbers, no conclusions can be drawn from the different mean lengths. The results are included, however, for comparison with the results from other rivers.

COMPARISON OF THE GROWTH OF SALMON AND SEA TROUT IN THE RIVER INNY.

In Tables 12 and 23 growth in the various categories of salmon and sea trout have been indicated. A complete comparison of the growth in the two species is not possible, but comparable groups can be compared, i.e. 2.1+ and 2.2+ groups in both species, (see Fig. 5). In every case the sea trout growth in fresh water was superior to that of the salmon, but at the end of the first year of sea life and at capture the salmon were superior in length to the sea trout. Similar conditions have been found in other rivers (see Went, 1941. *Proc. Roy. Irish Acad.* 47. B. No. 6. p. 174 and Went and Barker, 1943. " *Sci. Proc. R. Dublin Soc.* 23 (*N.S.*) No. 9. p. 97).

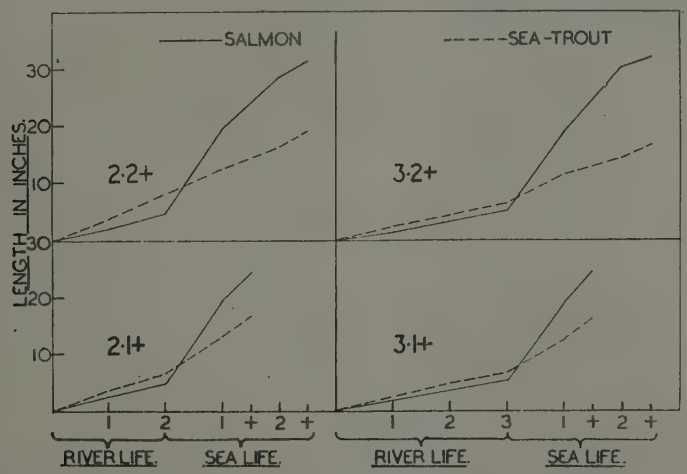


Fig. 5. Comparison of growth in salmon and sea trout in certain categories. (See above).

SUMMARY.

1. Material consisting of 747 sets of scales of salmon and 217 sets of scales of sea trout was collected during the years 1941-'44 from the River Inny (Fig. 1).

2. *Salmon.* Two-year smolts accounted for 80.6% of the total and three-year smolts for 16.3% (Table 3). The bulk of the fish were derived from type B smolts

(Table 4). The grilse formed 85.7 %of the total catch (Table 5) and the proportions of previous spawners (3.1%) and spring fish (4.2%) were small (Tables 5 and 7). A previous spawner which had spawned three times previously was noted (Plate 18). From the table showing divided migration and return (Table 8) it was shown that two-year classes contributed to over 96% of the stock. About 3/4 of the fish had lengths between 21.95 and 26.95 inches (Table 9 and Fig. 2). Average, maximum and minimum lengths of the various age groups were given in Tables 10 and 12. The proportion of fish having eroded scales and the mean degree of erosion increased as the season progressed. For the end of every year of life the lengths of each maiden or unspawned fish were calculated and the results are given (Table 11–12, 14–15). The fastest growing parr migrated first. (Fig 3.) Grilse were, on the average, longer at the end of the first year in the sea than any other age group (Table 11).

3. *Sea trout.* Two-year smolts formed over 3/4 of the total (Table 16) two smolt types being observed (Table 17). The fish were divided into the appropriate age groups (Tables 18 and 19). The question of divided migration and return (Table 20) was discussed. The average sizes of the different categories were given in Table 21. The oldest fish were in their seventh year. As in the salmon the growth of each maiden fish was calculated, and the results are given in Tables 22–24. Growth in fresh water was illustrated graphically (Fig. 4) when it was shown that the fastest growing parr migrated first.

4. A comparison of the growth in certain selected age categories of salmon and sea trout was made. (Fig. 5).

APPENDIX

Tables 3 to 15 relate to salmon and Tables 16 to 24 relate to sea trout.

Table 1. Chemical analysis of waters of the River Inny (results expressed as parts per 100,000).

Dissolved solids	(Volatile 1.6	Nitrates	0.066	Calcium Oxide (CaO)	0.7
	(Fixed 5.2	Chlorine	2.1	Magnesia (MgO)	0.1
Saline Ammonia	0.0015	Silica ($Si.O_2$)	0.8	Sodium (Na_2 0)	2.12
Albuminoid "	0.019	Phosphorus (P_2O_5)	Nil	Potassium (K_2O)	trace
Nitrites	0.01	Iron (Fe_2O_3)	less than 0.01		

Table 2. Monthly samples of sets of scales.

Month	Salmon		Sea Trout	
	No.	%	No.	%
April	3	0.4	56	26.0
May	41	5.5	97	45.9
June	102	13.7	29	13.7
July	510	68.3	23	10.9
August	63	8.4	2	1.0
Sept.	28	3.7	4	1.9
Total	747	100.0	211*	100.0

* No data for the capture of six fish.

Table 3. Distribution of the different smolt classes in the various age groups

Smolt Age	Age group (in winters)				Total for Maiden fish only
	1+	2	2+	With SMs.	
1	1.5	6.2	5.8	4.4	2.1
2	80.1	84.4	86.5	91.2	80.6
3	17.3	9.4	7.7	4.4	16.3
4	1.1	-	-	-	1.0
Total	100.0	100.0	100.0	100.0	100.0

Table 4. Distribution of the different smolt types in the various smolt classes.

Smolt Age	Smolt type				Total	
	A		B			
	No.	%	No.	%	No.	%
1	-	-	15	2.1	15	2.1
2	56	7.7	528	72.9	584	80.6
3	35	4.8	83	11.5	118	16.3
4	4	0.6	3	0.4	7	1.0
Total	95	13.1	629	86.9	724	100.0

Table 5. Percentage of each age group in the catch of each month.

Month	Age group (in winters)				Total
	1+	2	2+	With SMs.	
April	-	100.0	-	-	100.0
May	7.3	39.1	53.6	-	100.0
June	72.5	10.8	15.7	1.0	100.0
July	94.3	0.2	2.0	3.5	100.0
August	90.4	-	4.8	4.8	100.0
Sept.	89.2	3.6	3.6	3.6	100.0
Total	85.7	4.2	7.0	3.1	100.0

Table 6. Percentage of the total catch of each age group in each month.

Month	Age group				Total
	1+	2	2+	With S.Ms.	
April	-	9.4	-	-	0.4
May	0.5	50.0	42.3	-	5.5
June	11.6	34.4	30.8	4.0	13.7
July	75.1	3.1	19.2	78.0	68.3
August	8.9	-	5.8	14.0	8.4
Sept.	3.9	3.1*	1.9	4.0	3.7
Total	100.0	100.0	100.0	100.0	100.0

*A "dropper"

Table 7. Percentage of spring and summer fish in each month of the season.

	April	May	June	July	Aug.	Sept.	Total
Spring Fish	100.0	39.1	10.8	0.2	-	3.6*	4.2
Summer Fish	-	60.9	89.2	99.8	100.0	96.4	95.8

* One fish only a "dropper".

Table 8. Showing divided migration and return as percentages of the total catch for one year.

Returned in 1941 as	Hatched in the year					Total
	1935	1936	1937	1938	1939	
Grilse (1+ winters)	-	0.9	14.9	68.6	1.3	85.7
Small spring fish (2 winters)	-	0.4	3.5	0.3	-	4.2
Small summer fish (2+ winters)	-	0.5	6.1	0.4	-	7.0
Previous spawners (With S.Ms.)	0.1	0.3	2.6	0.1	-	3.1
Total	0.1	2.1	27.1	69.4	1.3	100.0

Table 9. The estimated size distribution of salmon as percentages of the year's catch.

Class Interval*	Age group (in winters)				Total
	1+	2	2+	With S.Ms.	
18	0.1	-	-	-	0.1
19	0.4	-	-	-	0.4
20	2.7	-	-	-	2.7
21	4.6	-	-	-	4.6
22	8.6	-	-	-	8.6
23	13.0	-	-	-	13.0
24	20.1	-	-	0.1	20.2
25	18.0	-	-	-	18.0
26	12.2	0.3	-	0.5	13.0
27	4.4	-	0.1	0.2	4.7
28	0.9	0.5	0.3	0.5	2.2
29	0.4	0.5	0.8	0.8	2.5
30	0.3	1.7	1.5	0.2	3.7
31	-	0.7	1.6	0.4	2.7
32	-	0.3	1.1	0.2	1.6
33	-	0.1	1.1	-	1.2
34	-	-	0.4	0.1	0.5
35	-	0.1	-	0.1	0.2
38	-	-	0.1	-	0.1
Total	85.7	4.2	7.0	3.1	100.0

* 18 etc. includes all fish between 17.95 inches and 18.95 inches etc.

Table 10. Average length in inches of the grilse in each month of the season.

Month	No.	Mean Length	Estimated weight *
May	3	24.3	5.7
June	63	23.2	5.0
July	508	24.7	6.0
August	41	25.0	6.2
September	25	26.3	7.3
Total	640	24.5	5.9

* On basis of K = 1.1 (see text)

APPENDIX—*continued.*

Table 12. Calculated lengths in inches for the end of every year of life in the different categories* in maidenfish only.

Category	River Life Lengths at end of				Smolt Length	Sea Life Length at end of		Length at Capture
	1st year	2nd year	3rd year	4th year		1st year	2nd year	
1.1+	3.3	-	-	-	5.0	19.7	-	24.8
2.1+	2.3	4.8	-	-	5.6	19.5	-	24.5
3.1+	1.9	3.7	5.4	-	5.7	19.4	-	24.7
4.1+	1.5	3.3	4.8	6.1	6.4	20.1	-	25.3
1.2	3.0	-	-	-	5.2	20.0	30.1	30.1
2.2	2.2	4.6	-	-	5.3	19.2	30.2	30.2
3.2	1.8	3.7	5.7	-	6.2	20.3	31.8	31.8
1.2+	3.0	-	-	-	4.7	18.5	29.8	31.9
2.2+	2.2	4.7	-	-	5.4	19.0	28.9	31.4
3.2+	1.4	3.4	5.2	-	5.8	19.2	30.1	32.2

* The symbol 1.1+ etc. indicates that a fish had spent one winter feeding in fresh water as a parr and 1+ winters feeding in the sea.

Table 11. Calculated lengths in inches in the sea for the various age groups.

Age Groups in winters	Smolt size	Sea Life Length at end of		Length at capture
		1st year	2nd year	
1+	5.6	19.5	-	24.5
2	5.4	19.4	32.4	32.4
2+	5.4	19.0	29.1	31.5
Total	5.6	19.5	30.3	-

Table 13. Monthly proportion of fish having eroded scales and average degree of erosion.

Month	Age groups (in winters)			Total
	1+	2	2+	
Proportion of fish having eroded scales				
April	-	0	-	0
May	0	12.5%	4.5%	7.3%
June	0	18.2%	18.8%	2.0%
July	1.0%	100.0%	10.0%	1.4%
August	33.3%	-	33.3%	31.8%
September	20.0%	100.0%	0	21.4%
Total	4.5%	18.8%	8.7%	5.5%
Degree of erosion (on Menzies scale)				
April	-	0	-	0
May	0	0.5	0.1	0.2
June	0	0.5	0.3	0.1
July	0.03	3.0	0.2	0.3
August	0.7	-	0.3	0.7
September	0.5	3.0	0	0.6
Total	0.1	0.5	0.2	0.13

Table 14. Growth in fresh water (in inches)

Smolt Age	Mean length at end of				Mean smolt length
	1st winter	2nd winter	3rd winter	4th winter	
1	3.2	-	-	-	4.9
2	2.4	4.9	-	-	5.6
3	1.8	3.7	5.4	-	5.7
4	1.5	3.3	4.8	6.1	6.4

Table 16. Distribution of the various smolt classes in the various age groups (previous spawners included).

Post migration year	Smolt age (in winters)				Total	
	1	2	3	4	No.	%
1st	-	9	5	2	16	7.4
2nd	12	152	25	4	193	89.0
3rd	1	5	1	-	7	3.1
4th	-	1	-	-	1	0.5
Total	13	167	31	6	217	100.0
%	6.0	76.9	14.3	2.8	100.0	-

Table 17. Distribution of the two smolt types in the different smolt classes (maiden and previous spawners included).

Smolt class	Type A	Type B
1	-	100.0%
2	9.5%	90.5%
3	32.3%	67.7%
4	33.3%	66.7%
Total	12.9%	87.1%

Table 18. Distribution of the various age groups.

Post migration year	Maiden Fish		Previous Spawners *		All Fish	
	No.	%	No.	%	No.	%
+	3	2.0	13	20.3	16	7.4
1+	143	93.5	50	78.1	193	89.0
2+	6	3.9	1	1.6	7	3.1
3+	1	0.6	-	-	1	0.5
Total	153	100.0	64	100.0	217	100.0

* Age at first spawning only considered.

Table 19. Monthly distribution of different age groups.

Age	April	May	June	July	Aug.	Sept.	Total
	-	-	-	-	-	2	2
1	34	66	22	14	2	2	140
2	1	2	1	2	-	-	6
3	-	1	-	-	-	-	1
With S.Ms.	21	28	6	7	-	-	62
Total	56	97	29	23	2	4	211 *
%	26.6	45.9	13.7	10.9	1.0	1.9	100.0
% Previous Spawners	37.6	28.9	20.7	30.4	0	0	29.4

Table 15. Growth in the different smolt types in each smolt class.

Smolt Age	Type A						Type B					
	No.	Mean length at end of				Mean Smolt Length	No.	Mean length at end of				Mean Smolt Length
		1st winter	2nd winter	3rd winter	4th winter			1st winter	2nd winter	3rd winter	4th winter	
1	-	-	-	-	-	-	15	3.2	-	-	-	4.9
2	56	2.3	5.3	-	-	5.3	528	2.3	4.8	-	-	5.6
3	35	1.8	3.8	5.8	-	5.8	83	1.8	3.6	5.2	-	5.6
4	4	1.5	3.3	4.9	6.4	6.4	3	1.6	3.3	4.8	5.9	6.5

APPENDIX—*continued.*

Table 20. Divided migration and return showing the years in which the fish were hatched.

Returned in 1944 as fish in their	Hatched in					Total
	1938	1939	1940	1941	1942	
1st Post-migration year	-	-	-	1	2	3
2nd ditto.	-	3	19	112	9	143
3rd ditto.	-	1	4	1	-	6
4th ditto.	-	1	-	-	-	1
Previous spawners	9	18	31	6	-	64
Total	9	23	54	120	11	217

Table 21. Mean lengths in inches in the different age categories.

Age Category	No.	Mean length in ins.	Age Category	No.	Mean length in ins.
2.+S.M.+	4	15.7	2.1+2 S.M.+	12	20.5
2.+2 S.M.+	4	18.5	2.1+3 S.M.+	5	21.2
2.+3 S.M.+	1	18.3			
			3.1+	19	16.5
3.+	2	12.8	3.1+S.M.+	3	17.8
3.+S.M.+	3	15.7	3.1+2 S.M.+	3	20.2
4.+	1	14.5	4.1+	3	16.9
4.+S.M.+	1	16.9	4.1+S.M.+	1	17.0
1.1+	9	15.5	1.2+	1	15.9
1.1+S.M.+	2	17.2			
1.1+2 S.M.+	1	20.1	2.2+	4	19.0
			2.2+S.M.+	1	20.3
2.1+	111*	16.4			
2.1+S.M.+	23	17.6	3.2+	1	16.6
			2.3+	1	17.5

* One not measured.

Table 22. Growth (in inches) in fresh water in the different smolt classes.

Smolt Age	No.	Length at end of				Mean Smolt Length
		1st year	2nd year	3rd year	4th year	
1	10	4.2	-	-	-	6.4
2	113	3.3	6.6	-	-	7.5
3	22	2.5	4.9	6.9	-	7.6
4	4	2.1	4.6	7.1	8.9	9.6

Table 23. Estimated growth in inches in the various age categories.

Age Category	No.	Years in River				Mean Smolt Length	Years in Sea		Mean Length at Capture
		1	2	3	4		1	2	
3.+	2	2.5	5.2	8.6	-	9.8	-	-	12.8
4.+	1	2.2	5.7	7.9	10.8	11.8	-	-	14.5
1.1+	9	4.2	-	-	-	6.5	11.9	-	15.5
2.1+	109*	3.4	6.5	-	-	7.5	12.6	-	16.4
3.1+	19	2.5	4.9	6.8	-	7.4	12.3	-	16.5
4.1+	3	2.1	4.4	6.8	8.3	8.8	13.4	-	16.9
1.2	1	4.0	-	-	-	5.8	9.3	12.7	15.9
2.2	4	3.8	8.1	-	-	8.8	12.5	16.1	19.0
3.2	1	2.3	4.4	6.7	-	8.0	11.6	14.3	16.6

* Three fish not measured.

Table 24. Estimated growth in inches in various age groups.

Age group.	No.	Mean smolt length	Years in Sea		Mean length at capture
			1	2	
+	3	10.5*	-	-	13.4*
1+	140	7.4	12.5	-	16.3
2+	6	8.2	11.8	15.2	18.1

* Only older smolt classes included (See text).

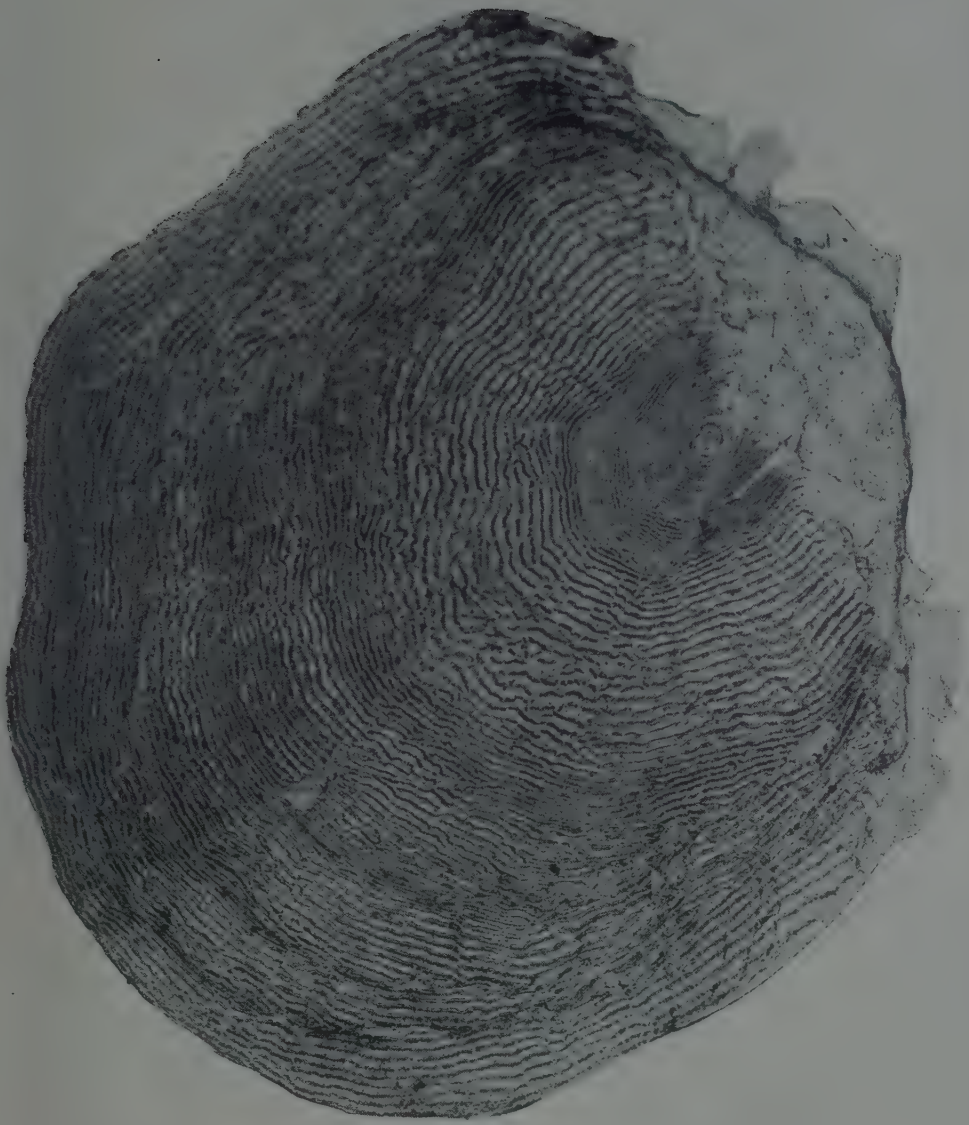


PLATE.—Photograph of a scale of a salmon (35 inches long) which had spawned on three previous occasions captured on 19th July, 1941. Magnification x 25.

No. 30.

THE DIAZO REACTION IN THE TETRAZOLE RING.

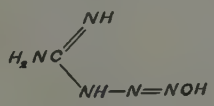
By

J. REILLY, J. P. TEEGAN AND M. F. CAREY.

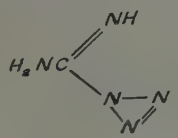
[Read MARCH 25, 1948. Published separately SEPTEMBER 29, 1948]

THE diazotisation of primary amines with the formation of diazonium salts is a characteristic reaction of practically all aromatic and many heterocyclic ring systems (1, 2, 3). For diazotisation, unsaturation *and* carbon attached thus :— $\geqslant CNH_2$ appear to be essential, *i.e.* unsaturated carbon attached to the amino group with a second centre of unsaturation in the complex. Even here some heterocyclic ring systems are exceptional. In the case of open chain compounds, aminoguanidine is in a unique position. In some ways it appears to be in line with aromatic primary amines, but the conditions of the reaction are quite distinct, with tetrazole ring formation in some cases.

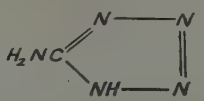
The normal method of diazotisation with sodium nitrite in the presence of free mineral acid yields, with aminoguanidine salts, salts of a compound considered by Thiele (4) to be " diazoguanidine."



but which subsequently was shown by Hantzsch and Vagt (5) to be guanyl azide :—

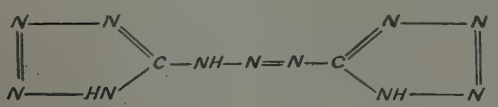


This azide on treatment with a weak base isomerises to 5–aminotetrazole :—



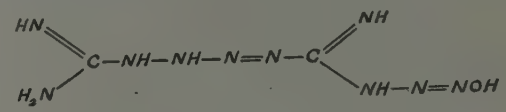
which on diazotisation gives a diazonium salt that couples with components such as α – and β-naphthylamine and β-naphthol, as was noted by Thiele (4).

Replacement of mineral acid by glacial acetic acid leads to the formation of 1–3 ditetrazolyltriazene :—

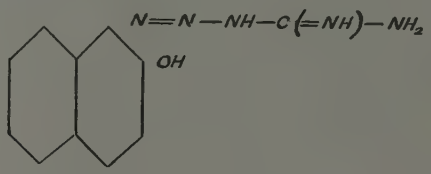


which was shown by Hofmann and Hock (6) to give coloured solutions with intermediates. In the thermal decomposition of 1–3 ditetrazolyltriazene, these authors obtained nitrogen with a small amount of cyanogen. The latter substance is probably due to working with a product contaminated with the open chain diazohydroxide.

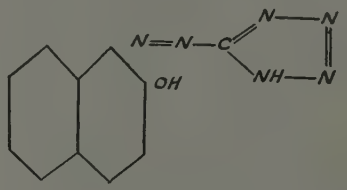
Hofmann and Roth (7, 8) have claimed that if aminoguanidine nitrate (or sulphate) in aqueous solution is treated with sodium nitrite under special conditions there results a true aliphatic diazonium hydroxide :—



This hydroxide is also claimed to couple slowly with α – and β-naphthylamine and m-phenylenediamine to give coloured (azo) solutions. The coloured compound from β-naphthol has been prepared and examined by Norris-Shreve, Carter and Willis (9) who gave the structure :—

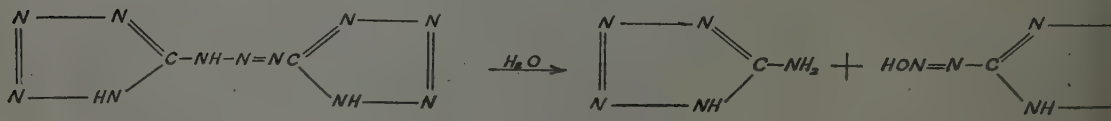


In the present work a comparison has been made between the β-naphthol derivative obtained from 1–3–ditetrazolyltriazene and that from Hofmann and Roth's diazonium hydroxide. Experimental evidence shows that they are the same compound, namely 1–2–3–4–tetrazole–5–azo–β-naphthol:—

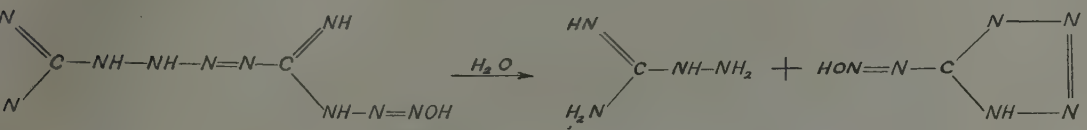


The same compound was also obtained by diazotising 5–aminotetrazole and coupling this with β-naphthol.

In the case of the 1–3–ditetrazolyltriazene, during the coupling reaction, which is carried out in aqueous solution at 80°C, it has been shown (see Experimental Section) that splitting occurs at the –NH–N=N– link with the formation of 5–aminotetrazole and tetrazole–5–diazonium hydroxide.



The diazonium complex was confirmed by the formation of an azo derivative. When no intermediate is present the diazonium hydroxide loses nitrogen, and forms 5–hydroxytetrazole. In the case of the straight-chain diazonium hydroxide it has been found that a secondary reaction is involved. Splitting occurs, as before, at the $-NH\dagger N=N-$ link, followed by ring closure and the formation of tetrazole 5–diazonium hydroxide :—



The tetrazole 5–diazonium hydroxide then couples with the β-naphthol in the normal manner. The azo derivative is therefore that of a heterocyclic-aromatic grouping and not an aliphatic-aromatic complex.

Preliminary experiments with the straight-chain diazonium hydroxide in inert solvents gave no evidence of intramolecular isomerism. This point however, is being further investigated.

The structure of the foregoing two β-naphthol derivatives was shown by reductive splitting with the formation of α-amino–β-naphthol and 5–amino-tetrazole.

EXPERIMENTAL.

Preparation of β-naphthol derivative from 1–3–ditetrazolyltriazene.

4 g. (.027 mol.) β-naphthol were added to 300 ml. of water and the whole heated to 80°C, which temperature was maintained throughout the entire experiment. 6.25 g. (0.027 mole) of 1–3–ditetrazolyltriazene (*disodium salt*) were now added slowly over a period of fifteen minutes with continual stirring. During the addition, a deep red colour developed accompanied by a small evolution of nitrogen. On cooling, unreacted β-naphthol was filtered off. 5 ml. of concentrated hydrochloric acid solution were added to precipitate the orange-coloured β-naphthol derivative. This was purified by recrystallisation from toluene (cf. Norris-Shreve *et. al.* (9).

In its general properties, this compound closely resembled that prepared by Norris-Shreve *et. al.* from Hofmann and Roth's diazonium hydroxide. It was almost completely insoluble in cold water, slightly less so in hot, the solution having an orange colour. It was insoluble in benzene, ether, chloroform. It dissolved readily in solutions of alkalis, even in such weak bases as pyridine, giving a red solution. M.P. (when recrystallised from toluene) :—165°C. Found ; C, 55.0 ; H, 3.0 ; N, 36.3 : $C_{11}H_8ON_6$ requires : C, 55.0 ; H, 3.3 ; N, 35.0.

A sample of the β-naphthol derivative as obtained from the diazonium hydroxide by Norris-Shreve, Carter, and Willis (8) was now prepared. The properties of this derivative coincided closely with those already given for that prepared from 1–3 ditetrazolyltriazene, and the mixed melting point was 165°C. Found : N, 35.6 : $C_{11}H_8ON_6$ requires N, 35.0.

The presence of one azo link in the molecule of the β-naphthol complex was determined volumetrically with standard titanous chloride solution.

Decomposition of 1–3–*ditetrazolyltriazene* (disodium salt) *on heating to* 80°*C. in aqueous solution.*

0.182 g. of 1–3–ditetrazolyltriazene (*disodium salt*) was heated to 80°C in 50 ml. water and the rate of evolution of nitrogen, with time, studied by collecting the gas in a water-cooled gas burette. As shown in Table I two atoms of nitrogen were lost per molecule of triazene, and the reaction followed a monomolecular course. The nitrogen evolved was free from cyanogen.

TABLE I.

Time (t)	Vol. $\begin{cases} 759 \\ 20° \end{cases}$	Vol. N.T.P.	K_1 (mono). 10^{-1}
1 min.	1–9 c.c.	1·76 c.c.	1·040
2 ,, ...	3·5 ,,	3·37 ,,	1·042
3 ,, ...	5·2 ,,	4·81 ,,	1·044
4 ,, ...	6·6 ,,	6·12 ,,	1·047
5 ,, ...	7·8 ,,	7·30 ,,	1·048
6 ,, ...	8·9 ,,	8·33 ,,	1·048
7 ,, ...	10·0 ,,	9·30 ,,	1·048
9 ,, ...	11·8 ,,	10·94 ,,	1·050
11 ,, ...	13·5 ,,	12·54 ,,	1·055
3 hrs.	19·2 ,,	17·90 ,,	—

This final figure of 17·9 c.c. corresponds with an evolution of one molecule of nitrogen from 1 molecule of 1–3–ditetrazolyltriazene (*disodium salt*) $C_2N_{11}HNa_2$ (0·182 mol. theoretically yields 18·1 c.c. at N.T.P.).

The resulting colourless solution was shaken with ether. The ether layer on evaporation yielded colourless crystals, which were identified as 5–hydroxy-tetrazole (M.P. 254°C). The aqueous layer, on evaporation, gave a residue which was recrystallised from alcohol, giving a crystalline compound M.P. 203°C. It was identical with 5–aminotetrazole, and a mixed melting point showed no alteration.

Reductive splitting of β-Naphthol complex.

This was effected, first, by using sodium hyposulphite. 0.3 g. of the complex was suspended in 100 ml. water at 100°C and 2.5 g. of powdered sodium hyposulphite were slowly added. Heating was continued until the orange colour due to the complex had disappeared. 5 ml. of concentrated hydrochloric acid solution were added which gave a precipitate. This was filtered off and shown to be *α*-amino -*β*-naphthol hydrochloride.

To a portion of the filtrate a solution of copper sulphate containing some sodium acetate was added, which gave a heavy green precipitate of the copper salt of 5–aminotetrazole (4). The remainder of the filtrate from the *a*-amino-*β*-naphthol was carefully evaporated, and crystals of 5–aminotetrazole hydrochloride separated. Reduction with tin and hydrochloric acid solution yielded the same products.

Preparation of β-Naphthol complex from diazotised 5–aminotetrazole and β-naphthol.

5–Aminotetrazole was prepared by diazotising aminoguanidine nitrate in the presence of free mineral acid, isolating the guanyl azide and refluxing this with sodium acetate solution. This base in solution was treated with dinitrogen trioxide in chloroform at o°C with constant stirring until it reacted to starch iodide paper. The lower chloroform layer was separated. The upper layer containing the diazonium derivative was treated with urea to destroy excess nitrous acid. A few drops of dilute hydrochloric acid solution were added. To this was added some *β*-naphthol dissolved in very dilute sodium hydroxide solution. An orange coloured precipitate developed. The compound, recrystallised from toluene, had M.P.=164°C. Found: N, 34.5. $C_{11}H_8ON_6$ requires N, 35.0.

1–3–ditetrazolyltriazene and Hofmann and Roth's diazonium hydroxide coupled at 80°C in aqueous solution with *β*-naphthol gave the same azo derivative.

An acknowledgment is made to the Industrial Research Council of Ireland for grants to two of the authors, J.P.T. and M.F.C.

1. Morgan and Reilly, Trans. Chem. Soc. (1913), *104*, 808.
2. Morgan and Reilly, Ibid, (1914), *105*, 435.
3. Reilly and Madden, Ibid, (1925), *127*, 2936.
4. Thiele, Ann. (1892), *270*, 1–63.
5. Hantzsch and Vagt, Ibid., (1900), *314*, 339.
6. Hofmann and Hock, Ber. (1910), *43*, 1866.
7. Hofmann and Roth, Ibid, (1910), *43*, 682.
8. Lieber and Smith Chem. Rev. 1939, *25*, 213—the section on The Action of Nitrous Acid on Aminoguanidine contains a summary of the work of Thiele, Hofmann, Roth *et al* on this subject.
9. Norris–Shreve, Carter and Willis, Ind. Eng. Chem. (1944), *36*, 426.

THE CHEMICAL DEPARTMENT,
UNIVERSITY COLLEGE,
CORK.

VOLUME 24.

1. Irish Previously Spawned Salmon. By ARTHUR E. J. WENT. 1s. (March, 1945).

2. Observations on *Gibberella Saubinettii* (Mont.) Sacc. on Cereals in Ireland in 1943 and 1944. By ROBERT MCKAY, D.Sc., and JAMES B. LOUGHANE, M.Sc. (Plate 1). 1s. 6d. (May, 1945).

3. Contributions towards an understanding of the Calcicole and Calcifuge habit in some Irish Plants. By D. A. WEBB, Ph.D., and ANNE V. HART, M.Sc. 1s. (June, 1945).

4. The Angular Distribution of Submarine Daylight in Deep Water. By H. H. POOLE. 1s. 6d. (July, 1945).

5. Developmental Lines in Pollination Mechanisms in the Coniferales. By JOSEPH DOYLE. 2s. (September, 1945).

[Nos. 1 to 5, price, 6s.]

6. Biochemical Analysis with the Spekker Absorptiometer. By EINHART KAVERAU, M.B., M.Sc. 1s. (May, 1946).

7. The Possibility of Initiating Thermo-Nuclear Reactions Under Terrestrial Conditions. By J. H. J. POOLE, Sc.D. 1s. (May, 1946).

8. The Mechanism of the Formation of Allophanates from Carbamates. By A. E. A. WERNER and J. GRAY. 1s. (May, 1946).

9. Dopplerite in Co. Limerick. By M. DEE and G. F. MITCHELL. 6d. (May, 1946).

10. Observations on the Pasmo Disease of Flax and on the Causal Fungus *Sphaerella Linorum* Wollenweber. By J. B. LOUGHNANE, R. MCKAY and H. A. LAFFERTY. (Plates 2, 3, and 4.) 3s. (JULY, 1946).

11. A Study of *Septoria Oxyspora* Penz. & Sacc. isolated from diseased Barley. By ROBERT MCKAY, D.Sc., (Plates 5, 6, and 7.) 3s. (JULY, 1946).

[Nos. 6 to 11, price, 9s.]

12. Studies on Ureides. Part 2. The Chemistry of Triuret and Related Compounds. By A. E. A. WERNER and J. GRAY. 1s. (July, 1946).

13. Evidence for a Mitotic Hormone: Observations on the Mitoses of the Embryo-sac. of Fritillaria Imperiatis. By HENRY H. DIXON, Sc.D., F.R.S. (Plates 8, 9 and 10). 2s. 6d. (July, 1946).

14. The Occurrence of Nickel and Magnetite in some Irish Serpentines, in conjunction with a Magnetic Survey. By D. W. BISHOPP, A.R.S.M. 1s. (July, 1946).

15. The Magnetic Properties of the Serpentine Deposits in the Slishwood Gap, County Sligo, Ireland. By W. E. SEEDS, M.Sc., and J. H. J. POOLE, Sc.D. (Plate 11). 2s. (August, 1946).

16. Award of the Boyle Medal to the late PROFESSOR THOMAS J. NOLAN, D.Sc., F.R.I.C.

17. On a recent Bog-Flow in Meenacharvy Townland, Co. Donegal. By W. W. BISHOP and G. F. MITCHELL. 1s. (October, 1946)

[Nos. 12 to 17, price, 7s.]

18. On a Recent Addition to the Collection of Irish Meteorites in the National Museum, Dublin. By H. J. SEYMOUR, B.Sc., B.A., M.R.I.A., Professor of Geology, University College, Dublin. (Plate 12.) 2s. (May, 1947.)

19. Irish Salmon, 1945. By ARTHUR E. J. WENT. 3s. (May, 1947.)

20. Salmon of the Kerry Blackwater. By ARTHUR E. J. WENT. 2s. (May, 1947.)

21. Effect of Mild Strains of Virus X on the Yield of Up-to-Date Potato. By PHYLLIS E. M. CLINCH and ROBERT MCKAY, Department of Plant Pathology, University College, Dublin. 2s. (May, 1947.)

22. Studies on Ureides. Part 3. (*a*) Constitution of Halwachs' Acid, (*b*) A New Copolymer of Cyanamide and Cyanic Acid. By A. E. A. WERNER. 2s. (July, 1947.)

23. Study of the Polymerisation of Cyanic Acid, with Special Reference to Dicyanic Acid. By A. E. A. WERNER and J. GRAY. 1s. 6d. (July, 1947.)

24. Notes on the Acidity, Chloride Content, and Other Chemical Features of Some Irish Fresh Waters. By D. A. WEBB, PH.D. 2s. 6d. (July, 1947.)

[Nos. 18 to 24, price, 12s. 6d.]

25. Award of the Boyle Medal to Professor J. H. J. Poole, M.A., M.A.I., Sc.D., 1947.

26. Transformation Reactions. I. The Mechanism of the Transformation of o-Aroyloxyacetoarones into o-Hydroxydiaroylmethanes, and the Synthesis of Flavones. By B. G. DOYLE, FIONNGHUALA GÓGAN, J. E. GOWAN, JOHN KEANE and T. S. WHEELER. 3s. May, 1948.

27. Seed Transmission of Virus Yellows of Sugar Beet (*Beta vulgaris* L.) and the Existence of Strains of the Virus in Eire. By PHYLLIS E. M. CLINCH and J. B. LOUGHNANE. (Department of Plant Pathology, University College, Dublin). (Plates 13-17). 6s. July, 1948.

28. The Chemical Constituents of Lichens found in Ireland. *Buellia canescens*. Part 3. The Constitution of Diploicin. By (The late) THOMAS J. NOLAN, JOSEPH ALGAR, EUGENE P. MCCANN, WILLIAM A. MANAHAN and NIALL NOLAN, University College, Dublin. 4s. (Sept., 1948.)

29. Salmon and Sea Trout of the River Inny. By ARTHUR E. J. WENT. (Plate 18). 4s. (Sept., 1948.)

30. The Diazo Reaction in the Tetrazole Ring. By J. REILLY, J. P. TEEGAN and M. F. CAREY. 1s. (Sept., 1947.)

[Nos. 25 to 30 Price 15s.]

PRINTED BY ALEX. THOM & CO., LTD. DUBLIN.